SCIENTIFIC PROSPECTUS

Sierra Dome Spider Research

Individual reproductive strategies in a changing microbial environment

Long-term research on *Neriene litigiosa*, begun at FLBS in 1980



Sierra dome spiders (*Neriene litigiosa*) on a natural web. Photograph: Paul J. Watson.

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Public scientific edition 1.1 | 12 November 2026

Purpose. To present the program's scientific foundation, attainable next steps, and long-term research opportunities to scientific colleagues, prospective assistants, collaborators, and successors.

Executive summary

Purpose and scientific opportunity. I have studied the complex sexual selection system of the Sierra dome spider (*Neriene litigiosa*) at Flathead Lake Biological Station (FLBS), in the field and lab, for more than 45 years. Research begun in this population in 1980, when I was still a University of Montana (UM) undergraduate, has produced nine major peer-reviewed papers in top journals on intrasexual competition, overt and cryptic female choice, multiple paternity, courtship, behavioral energetics, effects of disease, and offspring fitness outcomes. More papers based on already collected data are in the publication pipeline.

The spider has an annual life cycle and a breeding season that at FLBS lasts from mid-June through mid-August. Courtship, mating, fighting, and foraging occur in the middle of their dome-shaped webs conveniently placed exclusively in the forest shrub layer, almost all between 2 and 8 feet above the forest floor.

My many assistants and I have documented the reproductive lives of many hundreds of individual free-living females at FLBS. An important early discovery was that the processes of female mate and sire selection - separable because females control usage of the sperm they receive - begin well before a female's sexual maturation, during the late penultimate instar. Her first mate typically emerges from a championship series of 15-30 or more male - male fights. These occur due to determined multi-day male guarding attempts uniquely elicited by a special signaling behavior unique to penultimate females, which begins sometime during the week preceding maturation; onset of this signal, called associative behavior, strategically varies between females. Females invariably mate with the final champion male who manages to be present with them at the time of their final molt to adulthood and concomitant sexual maturation.

Later, secondary mates gain acceptance in a completely distinct way, namely, through pre-copulatory courtship entailing direct, elaborate intersexual strumming interactions with the female. If a female finds it in her interest to mate, actual insemination always is preceded by long and arduous copulatory courtship.

Most females mate with several males before their first oviposition. Meanwhile, however, females reject the vast majority of many other remating opportunities before egg-laying. Females fertilize their eggs as they are laid using sperm accumulated from their several mates, but their mates typically make very unequal contributions to the resulting brood.

The research has investigated signaling by both sexes, the information their signals convey, and how variation in signal quality influences decision-making during mating encounters and male - male fights. Its central aim has been to understand the adaptive significance of female mate and sire selection criteria, sequential polyandry, and the production of multiply sired broods. Published studies relate secondary males' fertilization success to body size and copulatory-courtship performance, and link nonrandom multiple mating to increased early offspring growth.

Laborious research in the 1980s on individually marked free-living females enabled multivariate analyses of diverse male traits and mating circumstances that successfully described about 30–35% of the variation among males in fertilization success. Subsequent behavioral energetics research precisely connected visible fighting and copulatory courtship performances to their heavy underlying, but highly variable, physiological and morphological demands. These findings opened, rather than completed, the question of what females learn about males and how that information affects male and female reproductive success.

The male personality studies begun in 2024 and 2025 have brought the investigation to an exciting new level. Repeated observations of nighttime exploration, courtship, and fighting are being used to build developmental life histories of individual males, revealing persistent patterns alongside flexible responses to different circumstances.

The near-term aim of the personality study is to understand individual reproductive neurodiversity through its expression in distinctive behavioral styles: complex, often fine-grained variations on species-typical and population-specific behavioral patterns. I want to understand how these male styles develop, how they fit with a male's physiological capacities, physical capacities, and microbiome. Importantly, I wish to learn much more about what females learn through behavior and chemical cues through courtship encounters with so many different male suitors before oviposition. Those behavioral histories provide the foundation for the next physiological, immunological, and microbial investigations.

With many modern, rapidly advancing molecular methods and new observational approaches in the field and lab, so much more remains to be done. I use "mating-system metagenomics" as an organizing term for my plans to dive deeply into how disease and individual microbiomes participate in reproductive development, performance, and female assessment. The question is not simply which microbes a spider carries, but how its immune system helps recruit and regulate them, and in turn how those associations potentiate or hinder what particular individuals can accomplish in the domain of reproductive competition, and how they accomplish it. Genetic and epigenetic regulation may help coordinate behavior, physiology, and microbial recruitment, bringing these components into useful alignment and helping to channel and stabilize the development of a "best feasible" individual reproductive approach.

I hypothesize that such development produces fuzzily bounded but recognizable "genres" of integrated behavioral, physiological, and morphological traits, within which individuals remain distinctive and flexible. Here, a genre means a recurring cluster of related male trait profiles, to be characterized through integrated measurements that include host-regulatory processes and microbial associations as well as behavior and body form. These are not uniform personalities or copies of one ideal male. I propose that they reflect evolved developmental strategies through which individuals make the best of the genetic, ecological, experiential, and - very importantly – the microbial symbiont cards they have been dealt through various life stages. Related organization may be more subtly expressed in females' patterns of mate and sire choice. I hypothesize that recurring, species-typical and population-specific requirements on the female side could help shape selection for the combinations of male capacities that repeatedly earn reproductive success.

A linked question is whether chemical cues and possibly signals help females interpret what an individual male's performance reveals. Odors arising from host physiology and microbial activity might provide contact or airborne "olfactory credentials" for the capacities underlying his distinctive behavioral style. This would connect both helpful and harmful microbial associations to what a male can perform, what he conveys during courtship, and his potential individualized contribution to offspring fitness. The benefit inherited by offspring could include a capacity to recruit and regulate useful microbial functions tailored to their genotype and ecological circumstances; offspring would not have to inherit their father's exact microbial community or personality.

This brings us back to the central puzzle. Most females mate with a few males while rejecting the vast majority of their secondary suitors, and the males they accept receive strikingly unequal shares of fertilizations.

Why these particular males, and why such different contributions? My working hypothesis is that female responses favor a bounded, nonrandom diversity of sires, not repeated copies of one superior male type. The value of an additional contribution may depend on the female's own constitution and reproductive health, her previous mates, the different demands faced by sons and daughters, and the ecological - including microbial - circumstances her offspring are likely to encounter. I hypothesize that these responses have evolved to maximize expected whole-brood fitness within the constraints females face. Understanding what the combinations and their relative contributions accomplish would help explain not only which males succeed, but why selection has favored the female's particular ways of choosing.

What makes these ambitions experimentally approachable is the unusual accessibility of the spiders' reproductive lives. In the FLBS studies, my assistants, collaborators and I have been able to concentrate on prolonged courtship and competition in fine detail on natural webs in relatively intact forest environments, recognize the responses of both participants, and work repeatedly with identified individuals. Decades of behavioral and physiological research have taught us how to ask increasingly revealing questions motivated and informed by maintaining contact with what the animals actually do in their untrammeled natural habitats. Modern molecular and chemical measurements can help explain the capacities and responses of individuals whose histories are intimately known. This is a radically optimistic but not unrealistic vision: the larger program can proceed through focused, connected investigations, each capable of yielding important results without requiring one student or collaborator to undertake the whole enterprise.

There is far more to investigate in this extraordinarily accommodating and tractable model system of sexual selection than I could ever hope to cover in my lifetime. I want to share the natural-history knowledge and methods developed through this work with investigators who can develop independent studies of their own. This prospectus sets out the existing scientific foundation and connected research opportunities for the next two or three decades.

Comparative studies of other Sierra dome populations would ask which relationships among development, microbial functioning, mate and sire selection, and offspring performance recur across settings and which depend on local circumstances. The observations accumulated at FLBS provide a starting point for these comparisons, including questions about variation in microsymbiont and disease ecology.

Younger investigators would inherit more than results and specimens. They would gain a well-developed living research system, methods learned through sustained practice, and the freedom to develop investigations I have not yet imagined. I welcome inquiries from prospective assistants, collaborators, and successors interested in these opportunities.

1. A living model of mate and sire selection

Evidence. Published findings, systematic firsthand observations, completed exploratory studies, preliminary analyses, and new hypotheses are distinguished throughout this prospectus. The natural-history observations draw on repeated work over many field seasons; unpublished does not mean casually observed. Comparative studies identify mechanisms and methods worth investigating here, not results already demonstrated in the FLBS population.

I have continued studying *Neriene litigiosa* because its elaborate reproductive behavior is exceptionally accessible to observation and experiment, both in the field and in the lab. Its web brings encounters into view without removing them from the setting in which they normally occur. We can follow what the male does, how the female responds, and how the encounter changes before either mating or rejection settles its immediate outcome.

The value of this long investigation is what those observations now enable us to ask. The program already connects natural encounters to competition, mating, fertilization, real-time behavioral energetics, and offspring performance. Each connection opens a further question. What capacities produced this performance? Why did this female respond as she did? And what consequences did the response have for the offspring that eventually developed? The next phase follows these questions into individual development and the microbial relationships that may help shape it.

Different routes to reproductive success

First mates and secondary mates enter a female's reproductive life by strikingly different routes. Before her final molt to adulthood, a female shifts from avoiding males to allowing close spatial association on her web. This "associative behavior" uniquely elicits multi-day male guarding attempts and a prolonged, sequential championship series of male–male fights, typically involving 15 or more contests. The duration of the tournament and the number of encounters it encompasses depend in large part on how long the individual female remains associative before maturing: 2.3 days on average, with an observed range of 0.5–5.0 days (Watson, 1990, and unpublished data).

Her first mate is the male present with her when she sexually matures—the "champ" remaining after the last contest, not simply a male she has encountered and accepted on the strength of his courtship alone. Her own associative behavior as a penultimate nearing maturation has brought about the stiff competition that determines who occupies that position. Females that mature during daylight hours invariably begin copulatory courtship and mating with the current guarding male, beginning 20–30 minutes after their final molt, as soon as their exoskeletons are adequately hardened. When females mature at night, the mating process typically begins at about 8–9 a.m. except in cold and rainy weather; no matings ever occur at night. In addition to being guaranteed a prompt mating without precopulatory courtship, first mates have been found to obtain about 70% of fertilizations, on average, in my earlier paternity analysis studies, although with considerable variation among females (Watson, 1991a,b).

Later, secondary males approach an already-mated female and generally gain their opportunities through direct precopulatory courtship rather than by winning another championship series. They must elicit her sexual receptivity through their web-strumming displays; obtaining her cooperation is a separate achievement from subsequently gaining a substantial share of fertilizations. The first mate therefore arrives at copulatory courtship with a competitive record already established, whereas a secondary mate has not passed through an equivalent championship on that female's web.

A field substitution experiment separated being the first mate from having earned that position through fighting. After a female matured, I replaced her champion with a small, competitively weak male. Females accepted these replacements, and the small males retained first-mate sperm precedence rather than yielding an increased share of fertilizations to later, larger males (Watson, 1991a). Thus the normal connection between championship success and first-mate identity could be broken without removing the first-mate advantage. This does not mean that size or competitive qualities are generally irrelevant to females. It means that first and secondary matings are different evaluative circumstances: the first-mate process cannot be represented as the same courtship-based assessment repeatedly applied to every male.

My repeated experimental observations clarify an important feature of the first-mate sequence. A sexually mature virgin female is fully receptive to an introduced male even when no championship has occurred on her web. The tournament is the usual natural route by which a male obtains that position, not an event the female must witness before she becomes receptive. This reliable receptivity also gives us a practical way to elicit a selected male's courtship and semen-loading sequence at a chosen time.

Copulatory courtship nevertheless subjects both classes of mates to an exacting performance test. My energetic measurements and close observations of hundreds of matings show that this is not a warmup or male sperm displacement behavior preceding insemination: vigorous performance severely taxes the male's metabolic machinery, while the repeated, precise operation of his elaborate paired mating structures—the palpi—tests developmental competence through their fit, coordination, and mechanical function.

The performance thus makes substantial information about metabolic and developmental competence directly available to the female. It may also reveal the functional consequences of illness and provide indirect information about immunocompetence. Copulatory courtship rigorously distinguishes between what a male is willing to attempt and what his body can actually sustain and execute.

These different routes to mating and sire success can be investigated in exceptional detail under natural conditions. In the earlier field studies, a closely coordinated team of three or four observers monitored the webs of 30–40 marked, free-living females. The organization and observability of the mating system make it possible to follow every intersexual encounter, not merely sample a few successful matings.

Males can be captured after completing a first or secondary mating; most unsuccessful secondary suitors can also be collected after their final rejection, clearly indicated by male behaviors characteristic of their preparation to leave the web to search for another female. We can therefore connect the behavior and subsequent examination of individual males to encounters whose unfolding, female responses, and immediate outcomes have been observed in nature (Watson, 1991a,b, 1993). These contrasting routes, and the unusually complete records that we can collect, provide the foundation for asking not only which males obtain mates, but what females learn about them and how that information leads to unequal sire success.

Winning a contest, securing a mating, and siring offspring are therefore not interchangeable outcomes. My studies relate secondary-male fertilization success to body size and copulatory-courtship performance, while identifying an elegant adjunct foraging enhancement function for female remating. The 1998 experiments linked nonrandom multiple mating to increased offspring growth rates during the spiderlings' most vulnerable 2nd - 4th instars (Watson, 1991b, 1993, 1998).

This provides a reproductive foundation against which new physiological and microbial explanations can be evaluated.

Separate stages make hidden mechanisms accessible

These different routes to mating and sire success become easier to interpret when we distinguish the successive stages of courtship and sperm transfer. Each stage gives the female access to different information and presents a different opportunity for her responses to affect the male's eventual reproductive success.

Precopulatory courtship functions to elicit female sexual receptivity through web-strumming displays before any genital coupling. Across my many years of field observation, females have most often mated with two to four males before their first oviposition, including the champion first mate. Some females, 16% at FLBS, accept no secondary mate, whereas mating with more than four males is uncommon. Females reject the vast majority of their secondary suitors, especially after their second mating. Multiple mating is therefore far from being an expression of indiscriminate receptivity: the few males accepted are greatly outnumbered by those whose courtship does not win them a mating.

The number of secondary suitors varies substantially among females and seasons. A female maturing late, after much of the male population has died, faces a different succession of visitors from one maturing near the beginning of the breeding season. My observations over many years consistently reveal numerous rejected opportunities alongside a small number of accepted secondary mates. That contrast, rather than a single rejection percentage or a fixed number of visiting males, is the important natural-history foundation for asking what females gain from the particular males they accept.

The structure of the mating system is such that almost all rejected males can be captured and preserved for later analysis, as can males who successfully complete first and secondary matings, even when working in the field with completely free-living individuals. This provides virtually unlimited opportunities to study diverse male characteristics that affect female sexual decision making.

Before beginning copulatory courtship, a male grooms and lubricates his pedipalps using a tiny, visible mucous droplet that accumulates at his mouthparts. This collectable secretion is distinct from semen and supplies another possible subject for chemical and microbial investigation, described in Section 5.

Aspermic copulatory courtship involves repeated coupling and uncoupling with sperm-free pedipalps, the paired structures - complex multi-component hydraulically powered moving machines - males use for energetically expensive genital coupling. Preinsemination phase copulation is another courtship performance, not insemination. After exhausting himself possibly to the point of exceeding his aerobic metabolic capacities, the male subsequently loads fresh semen into his pedipalps and returns for the **insemination phase**. The separation of these events is described in the published work (Watson, 1991b, 1993).

In encounters that proceed without female interruption (e.g., due to prey capture attempts, especially successful ones), males appear to determine when copulatory courtship ends. By all appearances, males perform to the point of absolute exhaustion, especially secondary mates. Nevertheless, unless they are actually near death, males never fail to build a sperm web, ejaculate, and charge their palpi with fresh semen immediately after cessation of copulatory courtship. This

transition is a conspicuous part of the reproductive sequence, with important experimental opportunities described below.

There is another aspect of copulation whose consistency is almost eerily absolute. In thousands of observed completed copulatory courtships, I have not seen a single female refuse the ensuing insemination, including after poor courtship performances; this invariant female “courtesy” remains somewhat mysterious to me, but may have evolved to maximally motivate exacting and arduous male copulatory courtship performances.

Permission to inseminate need not confer substantial paternity. Mate selection and sire selection are distinct; female-mediated discrimination over sperm storage or use is termed cryptic female choice.

My anatomical observations suggest a possible gating structure in the female sperm duct system that could regulate admission to storage. Its operation remains a hypothesis for modern imaging and functional tests, not an established explanation of all paternity patterns. It offers a concrete way to investigate how responses to a current male could influence eventual paternity without requiring the female to remember and rank earlier partners.

Copulatory courtship performance may also reveal developmental competence. I have encountered rare males that attain large body size, fight and perform precopulatory courtship effectively, but cannot couple their genitalia to females successfully, and others whose imperfect palpal fit impairs performance. Integrating behavior, anatomy, physiology, and parentage could distinguish willingness to invest in mating effort from the capacity to execute a successful display. That distinction is directly relevant to what females can learn about male health and inherited fitness-related capacities from male courtship.

Fresh semen from a selected male at a chosen time

The externally deposited semen is unusually accessible. I have repeatedly collected fresh droplets with an Eppendorf pipette in both field and laboratory settings. More importantly, collection can be deliberately elicited from a selected male rather than left to a chance observation. Once a male being followed through his developmental and behavioral history is sexually mature, I can introduce him to a mature virgin female on a suitably accessible web and obtain semen during the resulting reproductive sequence.

The timing is chosen by initiating that encounter, not by forcing ejaculation immediately. Copulatory courtship takes time, but its progression is familiar, and construction of the triangular sperm web gives an observer several minutes to prepare. A well-positioned field web beside a trail is especially useful: the pipette can reach the newly deposited droplet without passing through vegetation. A comparable captive arrangement can make the same collection possible.

The male makes an opening in the female’s dome and constructs his sperm web there. After depositing semen from the gonopore, he remains intent on taking it up into his pedipalps. The approaching pipette does not ordinarily cause him to abandon the task. That persistence makes collection practicable, but it also means that the collection fluid must be benign because he may contact it. Preservatives, when required for the intended analysis, are added after the sample has been removed from contact with him.

The normal sequence supplies two deposition opportunities. After taking up the first droplet, the male returns to the female and begins insemination-phase copulation. He then returns to the sperm web, or builds another, deposits a second droplet, and resumes insemination after charging again.

The two deposits can therefore be treated as distinguishable points in one male's reproductive effort, with their order recorded rather than presumed irrelevant.

This is an established collection method, not an established demonstration of microbes in semen. Although I have searched by microscopy and DNA-based approaches, I have not yet identified pathogens or microbial symbionts in the collected droplets. Under phase contrast, sperm and clear lipid-like or crystalline-appearing material are readily visible; the appearance of those other constituents does not yet establish their chemical identity.

The opportunity remains valuable whether or not intact microbes are eventually detected. Seminal constituents could be investigated in relation to the male's physiological history, sperm performance, or female responses. Their chemistry could also reflect host–microbe functioning elsewhere in the animal. The central advantage is access to material produced by a known living male at a selected point in his study, alongside a reproductive sequence we can observe in full. Future analyses would include controls for environmental and reagent background and account for the semen removed from the subsequent mating.

From male competence to offspring performance

The metabolic demands and exacting mechanical requirements of copulatory courtship make it a test of what the male's body can sustain and execute, not merely an impressive display. I expect substantial inherited contributions across the complex, polygenically influenced domains of metabolic competence, developmental competence, and immunocompetence. Those contributions are expressed through development, experience, and ecological circumstances, not independently of them. The important evolutionary question is which differences exposed by male competition and courtship contribute to the functioning of offspring. A female need not produce young that repeat their father's exact courtship style to benefit from selecting him as a sire. Capacities expressed in an adult male's reproductive performance may have their most consequential effects in the next generation during an entirely different activity and stage of life.

This was the motivation for measuring early offspring growth in my field study published in 1998. I chose growth during the first days and weeks of independent life, under standardized competitive foraging and web site selection conditions, as an integrated indicator of physiological performance, especially metabolic competence, with possible contributions from immune functioning as well. My field experience is that the vast majority of a generation is lost during these early weeks. Getting larger faster can reduce vulnerability to dehydration, starvation, and some forms of predation. A slightly larger spiderling can build a larger web, with a larger surrounding area of protection and warning. Early growth therefore changes more than body size: it changes the conditions under which the spiderling must obtain food and avoid becoming food. In this setting, even a modest advantage in growth could have a large consequence for survival. The point is not simply to grow into a large adult, but to survive long enough for any later reproductive advantage to matter.

In the experiment reported in 1998, I controlled female mating opportunities in nature and subsequently reared offspring under standardized conditions. Female mate number and the body mass of the known or most likely sire were positively and independently associated with early offspring growth after accounting for measured maternal and rearing effects. These results supported a genetic-benefits interpretation of female mating behavior (Watson, 1998). They supplied an existing connection between sexual selection in the parental generation and performance in the next—not a separate estimate of heritability for each physiological domain.

Early growth is an integrated outcome, not an isolated assay of metabolism or immunity. Its value here is that it allows the consequences of several interacting capacities to be expressed in a biologically important task under meaningfully competitive conditions. The next studies can ask which inherited contributions underlie that performance, how development and microbial associations affect their expression, and whether the same contributions carry different advantages or costs in sons and daughters or in different microhabitats. In this sense, “good genes” need not mean genes producing one universally superior male. The question is which inherited capacities improve offspring prospects in the circumstances they actually encounter.

2. Individual reproductive lives in unusually fine detail

Foundational field study

My previous exhaustive studies of behavioral and morphological traits of a female’s first and secondary mates allowed multivariate analyses that described about 30–35 percent of the variation among males in fertilization success (Watson, 1990, 1991a,b, 1993). The pivotal data set was based on 42 free-living females that my assistants and I monitored during the 1982-85 breeding seasons, on an hourly basis, through their late penultimate and adult lives: from the moment they became associative and began eliciting the championship series of fights that determine the first mate until oviposition, often two months or more later. We collected each of these females close to oviposition to obtain the egg case and offspring in captivity for the paternity analyses.

Many more females—hundreds—were observed during this phase of the study, but these exceptional 42 were those for whom we obtained the most complete reproductive histories and for whom the isozyme-based paternity analyses worked out. The steps these analyses require include many points of failure, including loss of many broods to disease.

Every male suitor accepted or rejected by these females was observed through the courtship and mating stages he reached and then captured for detailed morphological assessments. Males were then frozen for later paternity analyses. I would love to see this foundational study repeated using molecular methods that give more precise estimates of paternity for each of a female’s mates. But I am proud of this data set. Like the Watson 1998 follow-up study, the fieldwork behind it involved a monumental effort. Ultimate success was attributable to the exceptional observability and ease of handling of this spider species on a protected, near-pristine study site harboring a dense population, and to full access to FLBS microscopes, microbalances, and extensive general laboratory facilities. The facial and cheliceral photographs taken during those examinations provide a separate archive-based opportunity described in Section 5.

Yet I believe I still have not measured important female sire-selection criteria, potentially the most interesting ones of all. Describing 30–35 percent of the variation was an explanatory foothold, not the completion of the question. The next objective is to understand more of the differences among males in gaining a mating and, especially, in gaining fertilizations after mating. That requires following the male’s contribution more deeply: how he develops his capacities, how he deploys them during successive encounters, and how different females respond to what he offers. The potential for further fascinating study of this unusually accommodating system cannot be overstated.

Explaining male outcomes and selection on female preferences

The female-centered purposes of my early research career have always extended far beyond predicting which males win fertilizations. My archived NSF continuation proposal aimed to measure individual female mate and sire preference functions separately, and to then relate their differences to consequences for the female's own survival and fecundity, and the brood-specific viability and early instar competitive success of offspring of both sexes. The projected cross-generational tests were not completed as the original, half-funded, grant plan envisioned, but these questions remain fundamental (Watson, archived NSF continuation proposal).

A preference function describes how a particular female's responses vary with male characteristics and with her circumstances. It is therefore a characteristic of the individual female that can itself be studied, not merely a statistical description of the successful males. Within a population, most females are expected to have broadly similar needs that they address through mate and sire selection combined with moderate polyandry and production of multiply sired broods. No two females are identical in how best to use these species-typical, population-tuned tools. Two females could encounter a similar range of suitors and yet distribute mating acceptance and sire contributions differently. Explaining those differences requires asking what each evaluative pattern accomplishes for the particular female and her descendants.

The present personality and mating-system metagenomics program advances that same objective. A microbial characteristic that predicts paternity could help explain male success without, by itself, explaining why female responses to it are favored. We also need to know what those responses accomplish for the female and her offspring.

The bridge is therefore from observed male traits and reproductive outcomes to physiological capacities, individual developmental histories, the information available during courtship, and the fitness consequences of the female's evaluation. The new molecular and chemical work I have begun belongs within that evolutionary question, not beside it as a catalogue of additional measurements.

Behavioral energetics: from visible performance to physiological capacity

Behavioral energetics supplied a further step in that investigation. Seeing how rapidly or forcefully a male performs is not the same as precisely knowing the physiological effort involved, how long he can sustain it, or how his performance relates to the rest of his reproductive life. Precise measurements of the energetics of fighting and copulatory courtship capturing clean fine-grained variations in real time connect continually observed behaviors to the capacities the male brings to them. These measurements also enable examination of changing male decisions about shifts in the contexts into which they invest the most energy as they gain experience, age, or develop illness. Energetics research helps distinguish an impressive-looking action from the physiological work required to execute and maintain it and the reasons behind individual adjustments in mating effort. I hypothesize that young healthy males may be less judicious in their energetic expenditures than older males, who may increasingly save themselves for occasions where high effort can yield the greatest reproductive dividends.

The principal work on the behavioral energetics of successive fighting phases is published, notably the study with Tagide N. deCarvalho and Scott A. Field (2004). The paper with John R. B. Lighton (1994) was an important preliminary test of real time, flow-through respirometric measures of copulatory courtship energetics before I acquired my own TR3 system, hand made by Lighton's boutique company, Sable Systems. Because such measures must be adjusted for precise measures of

body mass, I simultaneously acquired a quality Sartorius M3P microbalance allowing reliable measures to the hundredths of a milligram.

The TR3 instrumentation is so sensitive and noise free that during periods when male and female are stationary and their combined resting rates are being recorded, a single momentary leg movement, for example, a simple grooming behavior involving one movement of one leg across a part of the body, produces a distinct reading of increased energy use that then declines quickly to baseline.

That exquisite system, acquired with support from a sole-PI NSF grant, supported many years of further behavioral energetics studies on the Sierra dome spider in addition to studies of bumblebee respiration patterns under changing hive conditions, the energetics of intersexual struggles in water striders, and the metabolic consequences of eating a seed to which subpopulations of soapberry bugs appeared locally adapted versus a nearby alternative seed source.

The additional copulatory courtship records link energy use, detailed behavior, body size, and, in portions of the archive, age, survival, or competitive performance in nature. The final report for NSF Award IBN-9321326 documents complete measurements on approximately 165 males during 1994–1998, a period encompassing the single award year, the no-cost-extension year, and subsequent unfunded years supported at my expense. These measurements were taken under standardized conditions, with two to five repeated measurements for many males. They include male and female resting metabolic rates, records of undisturbed complete copulatory courtship immediately followed by sperm web building, and complete records of insemination phase copulation. Many samples of pre- and post-copulatory records of male and female locomotion and grooming also are present in these records that offer good comparisons of energetic demands of routine non-courtship behaviors.

Another strength of these recordings arises from the fact that copulatory courtship involves *only male effort* while the female's resting metabolic rate remains stable and equal to her resting rate. Therefore, once that is subtracted, clean real time recordings of male energy use during courtship are available. Copulatory courtship requires that the female remain still. If she does not, she does not receive a fair and standardized assessment of the male's courting ability, because the pair's alignment must remain perfect for the male to attach a pedipalp. Female stillness also is selected for by predators. Any misalignment makes it hard or impossible for the male to detach a palp locked onto the female. The spiders have not evolved any cooperative means of defense or escape from any predator when their genitalia are coupled, and all their predators attack with great speed (wasps and birds are the major daytime predators, but also other spider species). Moreover, early in my copulatory courtship energetics studies, I performed a number of trials where I quickly removed the male from the respirometry flask in the midst of courtship, but leaving the female inside; this can be done efficiently with practice and luck combined. Success also is aided by using a high CO_2-free air flow-rate to purge the re-closed flask of intruding room air quickly. Considering only well-executed trials (N = 6), females showed identical resting and post-interruption metabolic rates.

This is a substantial, already collected physiological investigation awaiting fuller analysis and publication, not simply an aspiration to obtain repeated measurements. Season-specific records and individual links must be indexed before the larger collection is treated as a single analytical dataset (NSF final report; Appendix B).

Within that history, the archived proposal reports a 1994 repeated-measures study of 24 males of known adult age, observed in 68 courtships: two to four trials per male, usually several days apart.

Encounters used virgin females and were stopped after completion of aspermic courtship, before semen transfer, so that the receptive condition associated with uninseminated females could be maintained.

The resulting records separated enduring individual differences from age-related change under standardized holding, feeding, and mating opportunities. They are an analyzed component of the earlier work, not a second 165-male dataset or an equivalent of the recent multi-context personality cohorts (Watson, archived NSF continuation proposal, PDF p. 2).

An unusual feature of the system makes copulatory courtship especially informative. Male body mass varies greatly—from approximately 6 mg to more than 28 mg in my observations—but successful genital coupling requires every male's pedipalps to meet tightly constrained requirements of fit and mechanical operation. The copulatory apparatus cannot simply scale with the male's overall body size. Males of very different sizes and metabolic capacities therefore face a comparatively standardized performance task. How rapidly, accurately, and persistently each male operates their hydraulic palpal machinery can reveal physiological and developmental capacities that body size alone does not disclose. This helps explain why a female's joint assessment of size and copulatory performance could reveal advantageous combinations of metabolic power and energetic efficiency. The comparison concerns a standardized mechanical task, not an assumption that every male expends identical energy in accomplishing it.

Metabolic competence was deliberately examined as a combination of capacities. Table 1 of the archived proposal distinguishes maximum metabolic rate, relative energetic efficiency, scope above resting metabolism, the fastest increase in metabolic output, the stability of that increase, stability during sustained active performance, and resting metabolic rate. These are different ways in which a body can perform well or be limited; they need not place every male in the same order.

Maximum metabolic rate was defined using the highest three-minute mean. Relative efficiency concerned the energy expenditure required for a given rate of successful intromissions, not simply low expenditure regardless of what the male accomplished. The distinction matters because a male can attain a high output at a cost that another male does not bear to the same extent. The stability and speed of mobilizing that output add further dimensions to the physiological comparison.

Several measures showed repeatable differences among males, including an age-adjusted repeatability of approximately 0.48 for maximum metabolic rate, although not every measure was equally repeatable. Successful genital couplings and unsuccessful attempts were recorded separately. Physiological output can therefore be distinguished from attempted movements and from the consequences of imperfect palpal fit (Watson, archived NSF continuation proposal, Table 1 and Fig. 6).

The earlier analyses also motivated a question about the relationship between power and efficiency: whether male traits favored by females identify combinations that relax an apparent tradeoff between the two. Body size and copulatory-courtship performance provide complementary information. Body mass was positively associated with relative energetic efficiency, whereas intromission rate was positively associated with maximum metabolic rate—the male's metabolic "power." Body mass and intromission rate were themselves essentially uncorrelated. Power and efficiency, however, were negatively associated in the respirometry study, as illustrated in Figure 1 of the archived NSF continuation proposal. A male's strength in one physiological capacity therefore did not necessarily imply strength in the other.

One particularly intriguing, unpublished result suggests that females cross-reference these male traits when determining the fertilization success of secondary mates. The three-dimensional graph in Figure 2 of the archived proposal relates combinations of estimated metabolic power and relative energetic efficiency to observed fertilization success in 55 secondary mates studied in 1982–1985. Its shape is central to the biological interpretation: it depicts how reproductive success varies across combinations of the two capacities, rather than presenting each capacity as an isolated predictor. My interpretation is that females use size and copulatory performance together to favor physiologically advantageous combinations that partly overcome the apparent power–efficiency tradeoff. The analogy is choosing a car that combines strong high-speed passing ability with good fuel economy, rather than maximizing either capacity alone.

The graph brings together exceptionally labor-intensive field observations and paternity records with physiological relationships established through later respirometry. Body mass, intromission rate, and fertilization success were measured directly in the 1982–1985 males. Their maximum metabolic rates and relative energetic efficiencies were subsequently estimated from age-adjusted relationships measured in a separate 1994 study, rather than measured directly in those same males. The result therefore supplies a physiological interpretation of the observed reproductive pattern that awaits direct verification using physiological and paternity measurements from the same individuals (Watson, archived NSF continuation proposal, Figs. 1–2).

That obviously is a precursor to the present interest in coordinated capacities, not evidence that the genres or microbial mechanisms have already been demonstrated. It is also a substantial opportunity for a successor. I do not plan to collect additional behavioral-energetics measurements as part of my own continuation of the field program; my remaining research time must be concentrated on the current behavioral, developmental, and microbial investigations. The substantial energetics records already collected remain available for further analysis and publication. New respirometry could allow an interested successor to examine the power–efficiency relationship more directly, but it is not a prerequisite for the main work I am now proposing.

My respirometry system is carefully stored but returning it to service would require fresh calibration of key instruments, replacement of tubing, sufficient space for the extensive apparatus, and many days of hands-on training to obtain reliable, biologically interpretable measurements. Preserving the equipment and the practical knowledge required to use it gives successors an opportunity, not a ready-to-operate facility. A future investigator could evaluate refurbishment against newer instrumentation, with the choice depending on measurement requirements, technical support, and funding.

Early metabolic performance and subsequent physiological durability

Repeated measurements revealed something beyond repeatability. In the proposal's preliminary 1994 analysis, 13 males with demonstrated multiple-fight-winning histories showed faster declines in maximum metabolic rate during copulatory courtship than 10 randomly collected males; the reported comparison was $P = 0.016$. They also showed a more rapid but less dramatic decline in standardized resting metabolic rate. The two groups did not differ significantly in body mass, although in larger samples, champions do outweigh randomly collected males (Watson 1990).

Individual trajectories illustrated the same question, and among males with at least three measurements, higher initial maximum metabolic performance was associated with a steeper subsequent decline. These are unpublished results reported in the archived proposal, not new

analyses of the raw traces or established genetic causes (Watson, archived NSF continuation proposal, Figs. 3–4).

The observed decline concerns metabolic performance precisely measured by respirometry. It is not simply reduced visible vigor caused by imperfectly formed or poorly fitting pedipalps. My additional observations also suggest that males with the strongest early metabolic performances tended, on average, to have somewhat shorter lives. That longevity observation should be distinguished from the proposal's quantified metabolic-decline result.

Together they motivate investigation of initial performance, rate of deterioration, and total lifespan as related but separate features of a reproductive life. A more modest early performance need not mean inferiority in every consequential capacity, and high early expenditure does not by itself establish the cause of later decline.

This was the empirical origin of my concern about sex-specific inherited consequences. A male, especially a strong and aggressive fighter, may obtain a substantial prospective reproductive return early in the breeding season, whereas a daughter must sustain healthy functioning through prolonged egg production and oviposition. No females obtain anything close to early or even mid-breeding season reproductive success. If some inherited determinants that help produce exceptional early male performance also compromise later durability, their consequences could differ sharply between sons and daughters.

That cross-generational relationship remains to be tested; it cannot be inferred simply by equating a father's physiological trajectory with his daughter's. The hypothesis grew from measured male trajectories and the contrasting reproductive demands of females, not from an assumption that strong fighters are generally poor sires.

The value of this work for the present direction is not simply that more physiological data remain to be published. It establishes a way to connect outward performance with what an individual can sustain, and it supplies records with which to investigate relationships among effort, competitive ability, age, and longevity. The earlier paternity studies, the later behavioral-energetics collections, and the recent personality cohorts are separate datasets. They contribute different parts of the explanation; the next phase brings their questions together in new, deliberately linked individual histories.

The relationship to fighting also has structure. Body mass is a strong predictor of fight-winning ability, whereas copulatory courtship vigor predicts fighting ferocity. The retained proposal notes report a positive association between intromission rate and aggressiveness among males competing at a minor to moderate weight disadvantage, but not among their heavier opponents.

Here the behavioral characteristic concerns how hard a male fights when disadvantaged, rather than whether he wins. The distinction between competitive outcome and manner of competing is essential to the personality program: mass, ferocity, courtship performance, and contest success can be associated without being interchangeable measures of one general superiority (Watson, archived NSF continuation proposal, background and working notes, PDF p. 28).

Fast forward through many additional years of study to today's main questions, motivated and informed by far more research than can be summarized here. Knowing the cost of a performance and the outcome of an encounter still does not fully characterize the male who produced it. We need to understand the individuality he brings from one encounter to the next, and whether that

individuality helps explain why his contribution is accepted, rejected, or given a larger or smaller role by a particular female.

Males have different reproductively relevant personalities

In a creature like the Sierra dome spider, behavioral repertoires exhibit definite stereotypical patterns. It is easy to think that, once you have seen a few fights or courtship interactions and matings, you know the species-typical patterns of behavior—and to a superficial extent, you do. Studying the system in more detail, you see that these stereotypical behaviors are performed with different levels of precision and intensity by different males. If you take the time to watch more interactions, you begin to see that some males are behavioral outliers and violate the species-typical rules in obvious ways. Gradually, after you have watched thousands of interactions, as I have been inspired to do in this species, you begin to see that no two male–male or male–female interactions are identical. At a fine-grained level the personalities of the interacting individuals differ subtly, and such differences are magnified, especially at key junctures, in prolonged interactions.

Variation at this fine-grained level probably has substantial consequences for reproductive outcomes. We know as humans that high-stakes interactions are full of complexities that can greatly, and sometimes rapidly, affect behavioral and relational outcomes. I hypothesize that this is also true for the Sierra dome spider, especially in reproductively relevant encounters. Differences in male personality may matter greatly to females as they decide whether to mate and how much fertilization success to allocate to any given mate. Years of observation make it clear to me that females pick up on male personality differences: it shows in the way they respond behaviorally to these male variations.

To explore the mating system more deeply and understand more fully what females can learn from male fighting and courtship performances, we need to know more than whether a given male succeeded in a particular intrasexual contest or precopulatory or copulatory courtship effort. In much of my earlier fieldwork, which concentrated on strategic female decision making, a male was observed in only one reproductive encounter. We now need to know how the qualities of his successive performances with different females, in nature, fit into his broader reproductive life: how he explores, responds to rivals, persists or adjusts during courtship, and changes with age.

My current male-personality study extends the mating-system research to this level of the individual. By observing the same males repeatedly, it asks which behavioral tendencies endure, how their expression changes with circumstances, and whether the resulting combinations help explain different reproductive approaches, which I hypothesize undergo an appreciable degree of individual optimization during each male's development, based on experience and the self-assessments that reproductive encounters make possible.

Here, personality does not refer to a single measure or an inflexible behavior, but to identifiable behavioral patterns that manifest across different reproductively relevant contexts. For example, a male may be quite distinctive in how he adjusts to sexual rivals of different sizes and fighting styles or more or less receptive or otherwise reproductively valuable females. The ongoing study's approach depends on recording a variety of repeated behavioral interactions in enough detail to connect an individual's successive performances—a task for which the Sierra dome spider's natural webs provide an unusually revealing setting.

Continuous observation on natural webs

Courtship and male–male interactions occur in the central region of females' highly transparent, dome-shaped webs. Suitable webs are abundant and conveniently located in the shrub layer of the dense, protected FLBS population. A known male can be introduced onto a natural web without replacing the characteristic setting in which behaviors evolved to occur with an unfamiliar arena. With appropriate captive care and practiced introduction techniques, familiar courtship or competitive behavior usually begins within seconds on the study site in completely natural conditions.

Each current field personality trial includes thirty minutes of intensive observation and generally takes about one hour from preparation to completion. Two trained observers use data sheets refined over many years. One maintains uninterrupted visual attention to the spiders; the other also observes but concentrates on recording actions, timing, graded intensity, and the partner's responses. The record concerns how a staged interaction develops, not merely whether a male wins or mates.

My assessment of male behavioral repertoire variation and style rests on sustained observations of thousands of encounters. Skilled assistants also quickly recognize individual males' distinctive patterns and delight in coming up with appropriate names for them. I carefully train them, however, to record what occurs in each new observation rather than be influenced by what they expect. We welcome surprises: a male may respond differently to a larger rival or to a female that becomes strongly rejecting. Personality implies repeatability in behavioral patterns, but individuals can suddenly deviate from expectations. It is fascinating to witness these instances and to discover why. We begin to appreciate that part of the repeatability, the stable individuality, that we are testing for can reside in a characteristic way of adjusting to identifiable albeit sometimes subtle contingencies or in response to experience, rather than in rigid behavior.

Weighing males helps construct the experiment

To investigate those characteristic adjustments, we need to do more than observe whichever opponents happen to meet. We need to know, and sometimes deliberately choose, the physical challenge facing each male.

A Sartorius M3P microbalance is used routinely to measure male body mass to 0.01 mg and select fighting opponents. Mass differences of roughly 15% commonly produce rapid resolution via a ritualized non-contact display (referred to as "Phase 1" in my papers). Close matching gives greater opportunity to observe prolonged contests including pedipalp wrestling and occasional escalation to de-ritualized true and sometimes deadly fighting; deliberate mismatches reveal richer responses to advantage and disadvantage. Mass is therefore part of experimental design, not just a variable added to an analysis afterward (deCarvalho et al., 2004; Keil & Watson, 2010).

The longitudinal study is already under way

The intensive 2024 and 2025 field seasons followed approximately 15–20 identified males per season across the two- to three-week maturation window and a range of body sizes. The three repeated behavioral domains are **nocturnal exploration**, recorded in a large camera-monitored enclosure; **precopulatory courtship and female responses**, observed in the field; and **male–male fighting**, also observed in the field. Earlier repeated-measures work during behavioral-energetics studies provides additional precursor material.

Note that we record nighttime exploration in the name of greater ecological relevance; in nature, males do the vast majority of their travel between female webs after dark to avoid predation from birds and wasps. They aim to end up on a prospective mate's web by daylight, when courtship and mating exclusively occur beginning about 8:00 a.m., depending on the weather, and ending by twilight. Our exploratory records are generated by a data acquisition system, fed by a proven dual infrared camera motion detection system that monitors male movement patterns with high sensitivity regardless of male size. Male exploratory arenas are in large insect cages containing a fixed arrangement of sticks.

Adult males wander too widely for reliable, repeated tracking as free-living individuals. We maintain them individually, provide water, and adjust feeding—preferentially with wild-caught prey—to keep each near his capture mass. Males are retained until death, longevity is recorded, and bodies are frozen promptly. Specimens are linked to behavioral histories, body mass, maturation dates, and other individual records.

The existing records provide compelling observational evidence of persistent individual differences. More observations per male will improve quantitative estimates of consistency, context sensitivity, and change with age. Adding precise measurements of vibrational signaling with laser vibrometry would give us more detailed information about precopulatory styles and female responses. These measurements would extend a study whose participants are already behaviorally familiar, not replace it with a set of anonymous specimens.

The principally end-of-life specimens provide a useful molecular starting point. Selected measurements during adult life can draw on fresh semen, the secretion used during palpal grooming, and excreted droplets obtainable from known males. These accessible materials offer opportunities for repeated sampling; their analytical interpretation, live-animal measurements of emitted chemistry, and the effects of hemolymph sampling require further work, as described in Section 5. This combination could help distinguish the associations present during effective performance from changes that emerge only during terminal decline.

The aim is not simply to replace body size or courtship vigor with a personality score. It is to explain why males with partly similar measured traits can have different reproductive outcomes, and why the same male may be evaluated differently by different females.

Individual behavioral histories provide the context in which physiological and microbial measurements become interpretable. A larger fraction of fertilization success explained would be valuable, but the deeper advance would be identifying the capacities, information, and female responses that generate those differences. Unexplained variation in the older analyses motivates these questions; it does not by itself establish which new mechanisms will answer them.

Together, the natural encounter records, behavioral energetics, and repeated observations of known males make it possible to ask whether differences in exploration, courtship, competitive performance, and longevity form coordinated features of an individual reproductive life. Describing those features is one task; explaining how they become organized is another. The developmental framework that follows asks how a male's capacities and history might come together in a workable reproductive approach, and how recurring patterns of female choice could help shape that organization across generations, that is, on an evolutionary time scale.

3. How individuality becomes a reproductive approach

A male's reproductive life cannot be understood fully by assigning him a score for aggression, persistence, or courtship vigor. Each of those measures describes something he does, but none tells us how the different parts of his behavior fit together, how they fit the body doing the behaving, or how they change as he learns what he can accomplish. The developmental question is about that organization. How does a male arrive at a way of competing and courting that makes effective use of his particular capacities and circumstances?

A small male need not benefit from behaving as though he could win every championship. A powerful competitor may have opportunities unavailable to him but may also pay costs that a less forceful approach avoids. Even two males of similar size can differ in their capacity to sustain effort, their sensitivity to a female's responses, or their willingness to continue a difficult encounter. The personality study makes these differences visible. The next task is to explain how they become parts of individual reproductive approaches rather than treating them as disconnected variations around an average male.

Consolidation without uniformity

Genetic and regulatory differences, including epigenetic processes, microbial associations, nutrition, developmental noise, and individual learning may all contribute to male diversity. I hypothesize that, shortly before maturation and during early adulthood, development partly consolidates these influences into coordinated reproductive approaches. This would bring behavior into a workable relationship with attainable capacities and with combinations that repeatedly receive reproductive returns. It would not make every male a better or worse version of one ideal.

The word "consolidates" is important. I am not proposing that development eliminates the effects of an individual history. A male's experience may help determine which approach becomes effective for him, and the resulting individuality can remain conspicuous. What becomes more organized is the relationship among his tendencies: when he presses an encounter, when he changes tactics, how he sustains a performance, and how these actions fit what he can actually accomplish.

Such organization can coexist with flexibility. A male might respond to a larger opponent differently from the way he responds to an equal yet do so in a manner that is recognizably his own. Where development also buffers a useful combination against perturbation, "conditionally canalized development" is an appropriate hypothesis (Waddington, 1942). Repeated adult behavior establishes consistency; demonstrating buffering requires the further observation that an organized developmental outcome is maintained despite relevant disturbances. The two questions can be investigated together without being treated as the same result.

Genres of male reproductive phenotype. I use "genre" to describe a recurring cluster of male reproductive trait combinations. These are whole-organism profiles, not personality categories based on behavior alone. The relevant measurements begin with the behavioral tendencies and characteristic responses we observe repeatedly in identified males, together with body size and other physical characteristics. As the program develops, they can include metabolic and immune capacities, the composition and functioning of microbial communities, and patterns of gene expression and epigenetic regulation. The hypothesis is that these features become organized into several recognizable combinations rather than varying independently or placing every male somewhere along one scale from inferior to superior.

In using “genre,” I am not proposing the existence of rigid categories of Sierra dome males that develop highly restrictive mating tactics—for example, males that only compete for first matings versus males that only pursue secondary matings. In Sierra domes, every male retains a mixed, opportunistic mating strategy. A serial championship winner can, in his way, subsequently court an already-mated female. Similarly, a male with limited fighting prospects can take an available first-mating opportunity. What I hypothesize develops is a coordinated constellation of behavioral, physical, physiological, and microbial capacities that helps each male make the best of the cards he has been dealt. The proposed advantage is a combination of traits that makes him reliably persuasive as a mate or sire to females for whom his offerings are particularly valuable, while preserving his ability to take a decent shot at other mating opportunities. Such a combination could yield greater lifetime reproductive success than being mediocre at everything. His characteristic organization influences how he competes, courts, and responds to circumstances without confining him to one route to reproduction. Genres would describe recurring forms of that organization amongst males. All males in the population retain a shared, flexible mating repertoire, albeit often with individual stylistic differences in its expression.

A genre would be recognizable through the combination, even though no two males express it identically. Males within a genre could share a broad pattern of capacities while differing substantially in the finer details of personality, developmental history, and physiological organization. Some individuals may resemble more than one genre. The expectation is nevertheless of recurring, measurable structure: clusters with distinguishable patterns and potentially overlapping boundaries, not an undifferentiated collection of unique individuals. More complete measurements should help reveal which similarities are consequential, which differences distinguish genres, and how much individuality remains within each.

There is an evolutionary reason to expect such organization. My working hypothesis is that, for most females, no single “Mr. Right” supplies the paternal contribution that would best serve her whole brood. A female can instead benefit from a selectively assembled combination of sires whose contributions differ in useful ways, with their relative value depending on her own constitution, previous mates, and ecological circumstances. Recurring requirements of this kind could help sustain selection for several broad male reproductive profiles. Individual development would then help a male make an effective version of one of those profiles from the genetic, experiential, and microbial possibilities available to him. The proposed genres are therefore not merely convenient statistical groupings: the research asks whether their recurrence reflects developmental organization shaped partly by the different paternal contributions females benefit from acquiring.

Consider what this would mean when a female meets two males whose courtship styles differ. They might nevertheless sustain related combinations of metabolic and immune functioning and therefore offer partly similar paternal contributions. Alternatively, two performances that look much alike might be supported by different physiological arrangements, with different implications for durability or offspring. The genre metaphor gives us a reason to look beneath surface resemblance without dismissing the behavioral individuality that led us to ask the question.

Males with different histories and capacities could therefore develop toward different, individually attainable reproductive optima rather than converge on one ideal phenotype. The proposed optimum concerns the reproductive phenotype as a whole: behavior coordinated with physiological capacities and expressed in ways that make effective use of the male’s opportunities. It is not an optimum for personality considered separately from the body expressing it, nor simply a higher score for aggression, persistence, or courtship vigor.

A package of traits that is effective and efficient for one male, helping him persuade females to grant him paternity over his adult life, could lead to net fitness costs and below-average lifetime fertilization success for another. For a third, that package might be developmentally unattainable or prove a reproductive dead end. Developmental organization could thus preserve individuality while improving the fit between a male's capacities, his reproductive approach, and the female preferences and competitive challenges he encounters.

The point is not that every difference from an archetype is an imperfection. Some differences may reveal constraints or incomplete adjustment; others may be effective solutions to the individual's circumstances. Two males need not follow identical developmental routes to arrive at related reproductive capacities. Nor must those capacities be packaged in sharply separated categories. The proposed genres might consist of overlapping families or broad regions of effective combinations, with recurring relationships among traits within and across them.

A small male can change the terms of a contest. To understand how body size and behavioral style work together, it is useful to examine combinations that initially seem mismatched. A small male behaving with exceptional ferocity might appear to be courting disaster by acting beyond his physical capacities. But judging him solely by his prospects in a conventional strength contest misses the possibility that his behavior changes the contest itself. Following the interaction closely can reveal how a seemingly disadvantageous combination becomes effective through the response it elicits from an opponent. The example also lets us ask whether a combination that works in one contest remains effective across a male's reproductive life.

A rare but repeatedly observed male phenotype makes this possibility particularly tangible. Over many years, my assistants and I have encountered small, exceptionally fierce males whom we informally call "psychopath males." Their behavior illustrates why an effective relationship between body size and competitive style need not take the simple form of large males fighting boldly and small males retreating. These small males can sometimes prevail not by overcoming a larger rival's strength, but by making the encounter too dangerous for that rival to pursue.

Such males tend to eschew conventional, "proper" ritualized fighting stages, for example, by repeatedly attempting sneaky leg bites during otherwise ritualized Phase 1 encounters. They also avoid or rapidly truncate normally safe, ritualized Phase 2 pedipalp wrestling, a direct test of strength in which they would be severely disadvantaged against a much larger rival.

During ordinary ritualized Phase 2 wrestling, the males, using meticulous cooperation, lock together with their pedipalps over one another's shoulders and the anterior surfaces of their mouthparts pressed together. This posture prevents biting because the fangs move only from side to side. While locked together, the males test one another's strength by attempting to push the rival up and through the dome of the web. The small male's departure from that posture changes more than the intensity of the encounter: it changes how the contest can be decided.

Winning a Phase 2 bout does not necessarily settle the contest. A defeated male may disengage, regroup, and return—sometimes after a delay—to initiate another Phase 1 encounter. He may lose again, or he may reverse the previous outcome. I have watched many intense contests involving repeated bouts of Phase 2 that nevertheless remain beautifully ritualized. It is rare for a male to return after three successive Phase 2 defeats; when he does, escalation into Phase 3, or de-ritualized fighting, becomes very likely.

Renewed encounters always begin with Phase 1, although these displays become shorter and escalation more rapid as bouts repeat. Phase 1 does more than provide another opportunity for assessment. It brings the males into the relative positions from which they can cooperatively engage in Phase 2 pedipalp wrestling. Its recurrence therefore has a mechanical and coordinative role as well as an assessment role. Even an abbreviated return to Phase 1 helps organize the next bout.

Psychopath males tend to escalate rapidly into de-ritualized fighting, whether by bypassing prolonged wrestling or abruptly abandoning it. In unritualized fighting, luck plays a far bigger role in victory. The fight is akin to a frenzied knife fight, but even more dangerous. The knives inject venom. More importantly, the first male to land a halfway decent bite almost always wins, with immediate incapacitation that I attribute primarily to loss of blood pressure.

The contrast with ordinary male restraint is remarkable. Most males avoid escalation to full-on de-ritualized fighting, often forsaking what would seem to be good opportunities to bite their rival as they disentangle from a bout of Phase 2 assessment. The loser is sometimes especially vulnerable, as he is often quite entangled in the dome, with the victor often free to move with agility just a millimeter or two below him. Exactly how tangled and unable to defend himself the loser is may be hard for the victor to assess. Nevertheless, the victor almost always permits disengagement, despite having no guarantee that this opponent will concede permanently.

Restraint is evident even before the males engage in a properly aligned display. A male intent on initiating Phase 1 may be immediately behind a rival who is turned away from him, with his fangs only millimeters from the exposed abdomen. A bite there could immobilize the opponent through a sudden loss of blood pressure, yet the male pressing for a contest does not take that opportunity. He is plainly motivated to compete. What he seeks, however, is engagement in the ritualized assessment, not an immediate injurious attack. Willingness to initiate a contest and willingness to initiate damaging combat are strikingly different features of his behavior.

The “gentlemanly” restraint of typical males well outside the psychopath type therefore involves foregoing substantial potential benefits. They are missing the chance to eliminate a rival who might return and to acquire a fantastically valuable meal: males killed in battle are always thoroughly consumed. The rarity of post-bout attacks is not evidence that killing offers nothing. It is especially informative because killing could offer so much.

Those potential benefits must be considered alongside the risks to the attacker’s survival and subsequent reproduction. An attack would be spiteful in the technical evolutionary sense if its net direct-fitness consequences were negative for both attacker and victim. My interpretation of the persistent restraint is that, under ordinary circumstances, the additional benefits of attacking usually fail to justify its expected costs. A male can protect his present competitive advantage and preserve future opportunities without making the outcome of a renewed, lethal encounter depend on who lands the first disabling bite. I infer that selection has strongly favored this restraint and may have canalized adherence to ritualized assessment across much of the population’s otherwise substantial male behavioral diversity.

In contrast, small psychopath males have developed in such a way as to lack almost all assessment decorum. They show a readiness, even an energetic enthusiasm, for pulling precisely that “surprise” escalation after a brief or imperfectly cooperative initiation of Phase 2—or, even more commonly, for escalating fully and suddenly directly from Phase 1.

Such behavior clearly can and frequently does induce a much larger rival to withdraw. It makes good sense. The larger male's response is part of what makes his conservative approach effective: he potentially has many other reproductive opportunities ahead in which he has a good chance of success given his size. In the locally dense populations characteristic of this species, another female, sometimes an unguarded one, may be only a few feet away. The value of remaining alive and able to use those opportunities provides a plausible ecological reason for declining an encounter whose danger has become disproportionate to its immediate reward.

But success in one confrontation is not success in a championship series. A male guarding a penultimate female must remain in contention until she matures, potentially through ten, twenty, thirty, or forty encounters. An approach that works by repeatedly inviting dangerous escalation also repeatedly exposes its user to injury, death, or displacement. I would not expect the small psychopath approach to fare well over an extended championship: sooner or later a large and fierce rival is likely to refuse intimidation and push the small male out or kill him. The same readiness for escalation that secures an occasional immediate advantage could undermine the survival and persistence needed to turn that advantage into a first mating. These accumulated risks could help explain the phenotype's rarity. Evaluating that possibility requires comparing its lifetime returns, including secondary matings, with those available through other approaches open to males of similar size and condition.

Frequency dependence could provide a further explanation for why this departure from ritualized fighting remains uncommon. A small, exceptionally fierce male may gain an immediate advantage when a larger opponent declines the risk of grappling and biting. If such departures became more common, however, their practitioners would increasingly encounter opponents equally willing to escalate. A fighting style that sometimes secures a relatively inexpensive victory through the other male's withdrawal could then lead more often to dangerous combat, with substantial costs in injury, mortality, and lost reproductive opportunities. I therefore suspect that this approach would be unlikely to replace predominantly ritualized fighting, even if it sometimes pays particular males to use it when it is rare. The comparison concerns the lifetime reproductive returns to males expressing these tendencies, including the opportunities they preserve or lose after the immediate contest.

The extent of ordinary restraint is important to that hypothesis. In my observations, most adult male contests end in Phase 1, and relatively few progress beyond Phase 2. Under relatively normal conditions in the middle of the breeding season, when males still have substantial future reproductive opportunities, I estimate that only about 8–10 percent of fights escalate to non-ritualized grappling and biting. These are not two independent activities: the grappling is directed toward obtaining an opportunity to bite the opponent. The ritualized phases allow males to contest access to a female while usually avoiding that dangerous transition. I hypothesize that the resulting preservation of future opportunities helps maintain strong selection for ritualized fighting, while leaving scope for occasional or individually characteristic departures whose value depends on the opponents and circumstances encountered.

Our current personality observations provide a way to examine the decisions underlying these differences. The data sheets record which male initiates Phase 1 and, when we can determine it, which male attempts to initiate Phase 2. The Phase 1 initiator is clear in roughly 80–90 percent of encounters. We can therefore distinguish a male who actively presses for assessment from one who responds to his opponent, rather than describe both simply as participants in an aggressive encounter. Even among males that do not merit our extreme nickname, repeated staged fights

reveal individuals that are consistently bold and ferocious against larger opponents. Their characteristic ways of initiating, sustaining, and changing an encounter are part of the personality we are trying to understand.

These males offer a concrete opportunity to investigate how and when an unusual combination of body size and behavioral tendencies develops, the circumstances in which it succeeds, and what it costs over a reproductive lifetime. Its developmental origin could involve inherited differences, conditional regulation and experience, developmental noise, or some combination. The repeated occurrence of the phenotype and its observable competitive effects give us a phenomenon to explain; they do not require us to assume in advance that it is an optimally organized alternative.

Comparisons with other populations could be particularly revealing. How consistently do males adhere to ritualization, and how does that adherence vary with the ecological circumstances that determine the value of present and future reproductive opportunities? I would predict that extreme psychopath males remain uncommon, but understanding variation in both their occurrence and ordinary males' restraint would be valuable even if that prediction holds.

Whether females subsequently favor or disfavor psychopath males, or males with other outlier trait combinations, as secondary mates and sires is a further question. My impression is that females often do not favor psychopath males, although certain females might. If such discrimination reduces their mating or fertilization success, it could add to the lifetime costs that help keep the phenotype rare. The explanation would lie in the reproductive consequences for males expressing the approach, not in a collective need to protect the population's fighting conventions.

The example thus distinguishes three questions that an isolated victory cannot answer: how the male's behavioral approach develops, what competitive opportunities it obtains and preserves, and what females ultimately make of the contribution he offers. Competitive effectiveness gives a male access to opportunities, yet it does not by itself tell us what his contribution is worth to a female or how often it is valued.

The female's problem concerns the whole brood

Why, more generally, might a female benefit from several kinds of sire rather than repeated contributions from one favored kind? The question takes us beyond the male's immediate competitive achievement to the lives of the offspring he sires.

My working hypothesis is that female responses favor a structured, bounded diversity of paternal contributions, not indiscriminate novelty or repeated copies of one superior male type. A particular female may benefit from more than one useful combination, even when those combinations share important strengths. Secondary sires need not be the opposites of the champion first mate.

The objective I have in mind is expected whole-brood fitness: the combined prospects of the female's offspring for surviving, developing, and eventually reproducing. Competitive sons and physiologically durable daughters are not separate goals pursued for their own sake. They illustrate different demands that enter the larger reproductive outcome. A benefit concentrated in a few offspring might be important, but it must be considered alongside costs or losses elsewhere in the brood.

The Sierra dome female provides no parental care to the independently living young. She cannot meet them, discover their individual difficulties, and adjust her treatment accordingly. Her influence is exercised earlier, through the resources and paternal contributions with which they begin their

lives. Her evolved sensory and physiological responses could use information about herself, the males she encounters, and the surrounding conditions to improve those starting prospects. No conscious forecast is needed for a conditional reproductive response to have consequences in a future generation.

This makes the distinction between the number of mates and the composition of sires essential. Acquiring another male is not automatically useful because he is different. His contribution must offer something of reproductive value in the circumstances that matter to this female and her offspring. Likewise, a capacity that is valuable in one sire could be partly redundant in an additional sire, depending on the benefits a combination actually supplies. These are hypotheses about the consequences of the mixture, not assumptions that genetic diversity is always advantageous.

Different reproductive schedules can change the value of inherited capacities

A male can secure prospective paternity early and need not remain alive until the female fertilizes her eggs during oviposition, potentially one or two months after mating. His death need not erase the sperm already deposited, although its reproductive realization still depends on her. A female cannot bypass the intervening investment. She must obtain and allocate resources, maintain viable developing eggs, and complete oviposition.

The preliminary behavioral-energetics findings in Section 2 give this contrast an empirical starting point. Males with exceptionally strong early metabolic performances, including demonstrated multiple-fight winners, showed faster subsequent decline in the archived analyses. If some inherited determinants that help produce those early performances also influence physiological durability, their consequences could differ between sons and daughters. A contribution that helps a son make an early reproductive return could impose a greater cost in a daughter whose reproduction depends on sustained functioning later in life.

That is a specific cross-generational hypothesis, not an assertion that strong fighters are generally poor sires. It could involve intralocus sexual conflict, in which the same inherited variation has opposing fitness effects in males and females. Experimental work in *Drosophila* supplies a precedent for such opposing reproductive consequences across sexes (Pischedda & Chippindale, 2006). Whether the physiological relationship observed in Sierra dome males extends into daughters remains a question for parent–offspring investigation.

The relevant female capacity is sustained reproductive functioning, not longevity by itself. In this population, a female can remain alive, look robust, and be beautifully gravid while her developing eggs are failing. The disease syndromes described in Section 4 make that distinction painfully concrete. The qualities that matter must support reproduction through its actual sequence of vulnerabilities; looking impressive at one point in adulthood is not enough to establish that they do.

Mixed paternity could provide useful combinations without the female assigning particular fathers specifically to sons or daughters. Her responses could alter the representation of paternal contributions in a brood containing both sexes, while their inherited effects are subsequently expressed in different bodies and circumstances. The evolutionary question is whether the resulting mixture improves the brood's combined prospects, considering its costs as well as its benefits (Watson, 1991a, 1991b, 1998). Section 5 asks a further, separable question: whether filtering among genetically different sperm within a sire's contribution could also change which paternal alleles and combinations reach sons and daughters.

A collection of sires has a composition and a weighting

In this model, the female's collection of sires has both a composition and a weighting. The question is not only which contributions are present, but how strongly each is represented. A substantial contribution from the champion first mate could be supplemented by smaller contributions from secondary mates that add something useful. Another female, or the same female in a different physiological state, might benefit from a different balance.

Responses to successive males could influence how much sperm is admitted to storage, how long it is retained, or how it is subsequently used. These are different opportunities within the reproductive sequence. The proportions in storage need not translate directly into the proportions of eggs fertilized, and paternity among surviving offspring can change further if offspring of different sires differ in survival. The study must follow those transitions rather than treating one sample of offspring as a complete description of the female's preceding responses.

The first-mate substitution experiment is particularly informative here. A female given a small, competitively inferior substitute for her champion did not compensate by increasing the later males' fertilization shares. Secondary mating therefore cannot simply be described as her diagnosis and correction of an inadequate first mate. She might still gain insurance from additional sires without detecting each failure of the championship procedure. The stronger proposal is that diversification is organized around particular combinations of capacities and their relative contributions, operating alongside the demonstrated first-mate advantage (Watson, 1991a, 1991b).

There is also a useful theoretical test of the diversification argument. If every sire had one fixed expected value per offspring, independent of the other sires and all relevant circumstances, a mixture would simply average those values. It could not improve on the best component of that simple comparison. The proposed benefit of a mixture therefore needs a biological explanation: for example, consequences that depend on the composition of the brood, or differences in vulnerability that keep its members from failing together under the same challenge.

This is where the older genetic bet-hedging question remains relevant. A sire combination might reduce the chance of near-total reproductive failure even without raising the arithmetic average performance of every brood. Such protection concerns variation among possible outcomes and its consequences over generations. Models of bet-hedging polyandry identify conditions under which these effects can favor multiple mating, rather than treating risk spreading as a universal benefit (Yasui & Garcia-Gonzalez, 2016). In the Sierra dome system, both improved expected performance and reduced shared vulnerability deserve tests. They are related possibilities, not interchangeable explanations.

Female individuality within recurring preference patterns

A female encounters a particular male, not an abstract representative of population-wide quality. His value to her may depend on her genotype, developmental history, physiological and immune state, microbial circumstances, previous mates, and remaining reproductive opportunities. I use a female's "preference landscape" to describe how her responses assign reproductive value to different combinations of male traits. Under the structured-diversity hypothesis, several combinations can be useful to her, rather than only one ideal male.

The value of a contribution can change while the male himself remains much the same. A female developing difficulty in sustaining healthy eggs might place greater weight on a capacity relevant to that difficulty. A previous mating might have supplied a contribution that changes the usefulness of

another similar male. The informative observation would be a predictable change in how she evaluates a characterized male, not merely a fluctuation in general receptivity.

Sierra dome females have substantial opportunities to express such differences. They occur in locally dense populations, and the long interval between the first mating and oviposition brings many suitors onto a female's web. My repeated field observations indicate strong seasonal variation in those opportunities. A female maturing late, after much of the male population has died, faces a different sample of contenders and secondary suitors from one maturing earlier.

What remains consistent is the selectivity: females accept only a small fraction of the males available to them, and accepted mates can make very unequal reproductive contributions. Their potential collection is not limited to one encounter with an arbitrary male, but neither is it an unlimited sample of everything that would have been useful. The opportunities and constraints are both part of the evolutionary problem.

Individual female preferences need not be scattered arbitrarily across all possible male combinations. Females share much of their reproductive organization and encounter recurring demands imposed by the species' life history. At a finer scale, the FLBS population and microhabitat may impose recurrent nutritional and microbial challenges. A female's own experience then modifies the value of contributions within this shared setting. I hypothesize that individuality in mate and especially sire choice is organized around these broader, species-typical and population-specific themes.

The proposed symmetry is not one female type matched with one male type. A single female may value several reproductive genres, and different females may share some favored combinations while differing in others. The corresponding female "genres" would be recurring patterns in the composition and relative value of useful sires, not mirror images of male personalities. Their expression may be subtle: females can look similar in willingness to mate yet differ in the subsequent reproductive success they confer.

A shifting but recurrently structured sexual-selection seascape

A fixed fitness landscape is a useful way to imagine that some combinations of traits have greater reproductive value than others. But the relevant conditions do not remain fixed. The idea of a fitness seascape makes that temporal change explicit: the selective value of a combination can rise or fall as its environment changes (Mustonen & Lässig, 2009). I use it here to describe changing reproductive opportunities without losing sight of the recurrent patterns that might favor recognizable male genres.

The most important word is not "shifting," but "recurring." An arbitrarily turbulent set of female preferences would not explain the persistence of organized male alternatives. My hypothesis requires enough repeated structure in the combinations favored, and in the consequences of favoring them, for selection to shape developmental responses across generations. The exact weighting of useful contributions can change while some broader requirements remain sufficiently regular to matter.

The seascape moves, but it need not become unrecognizable every time it moves. A change in local pathogens could alter the value of particular defenses. A female's prior matings could alter the marginal value of another contribution. Yet reproduction would still require offspring to survive their early independent lives and remain functional through the demands of their own reproduction.

Shared requirements and changing circumstances can therefore coexist within the same explanatory picture.

Favored regions need not be narrow peaks occupied by nearly identical males. They could be broad, overlapping regions or ridges containing several effective combinations of capacities. That leaves room for an individually distinctive male to express a useful reproductive genre without approximating one perfect specimen. It also allows different developmental routes to yield partly comparable contributions.

A female's evaluation and the reproductive opportunities available to males across the population remain different levels of description. A male can encounter several females. His eventual return depends on which females he meets, how they respond, the rivals he faces, and whether he survives to make further attempts. Individual female evaluations help generate that broader seascape, together with competition and other ecological pressures; no single female defines it.

Nor need the sexes agree about the outcome. A female's proposed benefit concerns what the combined paternal contributions accomplish for her brood. A male's return concerns his own descendants. He may be valuable enough to enter her collection without benefiting from the proportion of paternity that would best serve her. The correspondence between female requirements and male development can therefore be a strong evolutionary relationship without being a harmonious one.

Evolution shapes the rules; development works with the individual

The connection to development is indirect but biologically specific. Across generations, female preferences, male–male competition, and other selection pressures could favor developmental rules that tend to produce effective combinations. Within a male's life, those rules operate through the conditions he actually encounters: inherited capacities, nutrition, learning, reproductive experience, disease, and available microbial associates. The seascape describes the reproductive consequences of phenotypes; it is not a map that the male reads.

Repeated encounters may nevertheless provide useful information. A male experiences opponents he can or cannot displace, performances he can or cannot sustain, and females whose responses change as an interaction develops. I hypothesize that such experience can participate in adjustment of his reproductive approach. The evolutionary history of the developmental system may bias those adjustments toward combinations that have repeatedly worked, while the male's particular history shapes their expression.

This is more than bringing behavior into line with a fixed body. Neural, metabolic, and immune regulation may develop in relation to one another. Microbial functions could expand what the male can sustain, while his regulatory capacities affect which microbial relationships can be maintained. The result would be an improving fit among behavior, host physiology, and microbial assistance, with each changing the possibilities available to the others.

Ecological conditions act on both sides of the encounter. Prey, nutrition, pathogens, and available microbes can alter what males develop and what females benefit from acquiring. The evolutionary relationship can also be reciprocal: changes in the male phenotypes available, and in their consequences for offspring, can affect which female assessment mechanisms are favored. The resulting "dance" connects individual development with selection across generations. It is structured by repeated biological demands without prescribing identical steps for every individual.

Female mate and sire choice may therefore favor more than the capacity to develop without error. It may also favor inherited regulatory capacities that help an individual develop appropriately: coordinating behavior, physiology, and microbial relationships in ways that make effective use of its own constitution and the circumstances it encounters. Where these capacities improve offspring survival and eventual reproduction, they could increase the choosing female's own reproductive success. Across generations, such choice could help keep the genetic basis of developmental responsiveness "updated," that is, aligned with recurring but changing ecological and reproductive demands.

From a compelling explanation to discriminating tests

The hypothesis has concrete tests. Do early condition and experience predict later combinations of behavior and physiology? Does coordination become more evident near maturation and during early adulthood? Do particular combinations obtain different mating or fertilization returns with different females, and do those differences recur under comparable circumstances? Comparative studies already show the value of investigating developmental history and the fit between phenotype and context, while leaving the Sierra dome mechanism to be established (DiRienzo & Montiglio, 2016; Montiglio et al., 2017).

These questions can be investigated by constructing multivariate profiles of individually known males. Repeated observations would characterize not only average behavioral tendencies but also characteristic adjustments to different opponents and females, while physiological and molecular measurements progressively extend the profiles. Cluster analysis, including approaches that allow graded membership, would help assess whether recognizable combinations recur across individuals. Principal-component analysis or related methods could help summarize the major dimensions of variation. The objective is to identify the defining combinations, the variation within them, and the extent of overlap among them—not to decide in advance how many genres the spiders must possess. The analyses would also compare the proposed recurring, potentially overlapping genres with the alternative that male traits vary in coordinated ways without forming reproducible groupings.

Candidate genres would be characterized from the males' measured traits and then examined for their developmental and reproductive significance. Do related profiles emerge through comparable developmental processes? Do they recur in additional males and seasons, and how do their expression and prevalence change with ecological circumstances? Most importantly, do particular profiles receive different mating and fertilization returns from females with different histories and existing sire contributions? Identifying a recurring cluster would establish a pattern to explain. Connecting it to development, female evaluation, and offspring outcomes would test why that pattern matters.

This provides a clear transition to mating-system metagenomics. We have an observable behavioral problem and an evolutionary account of why several organized solutions could matter. We now need to investigate how an individual assembles the capacities behind his solution, and what information allows a female to judge the contribution he offers. Epigenetic regulation and host–microbe relationships belong at that point in the explanation, where they can help connect a life's history with the performance of a living animal.

4. Mating-system metagenomics: development and assessment

The developmental framework raises two connected questions. How does an individual male build and sustain the combination of capacities he brings to a reproductive encounter? And how does an individual female assess what that combination could contribute to her offspring? Microbial associations may participate on both sides: helping to construct the male phenotype and contributing information through which a female interprets it.

I use "mating-system metagenomics" as an organizing term for this integrated investigation. Sequencing can identify microbial associates and their potential functions, but the scientific aim is larger than an inventory. It is to understand how the microbial ecology of this spider population *participates* in development, reproductive performance, female mate and sire selection, and the fitness of offspring. Pathogens and useful microbial associates belong in the same evolutionary account because (1) they may interact in ways that affect the host, and (2) both can change what an individual is able to do and what another individual can learn about it.

Rowe and colleagues (2020) provide a broad evolutionary framework linking the reproductive microbiome to sexual selection, sexual conflict, mating systems, and reproductive isolation. Their important review considers both beneficial and harmful microbial functions, compatibility between reproductive partners, sperm competition, and cryptic female choice. It also emphasizes that the relationship runs in both directions: microbes can influence reproductive interactions, while mating changes the opportunities for microbial transmission, establishment, and evolution. This framework provides an important conceptual foundation for the investigations proposed here.

The particular developmental extension I want to pursue concerns how host–microbe relationships participate in the organization of intrasexual reproductive neurodiversity. A male's distinctive behavioral style develops in a body with particular physical capacities, physiological possibilities, and an individual history. I want to understand how those components, including microbial assistance and its regulation, become coordinated into the recurring yet individually variable reproductive combinations described in Section 3. The corresponding female question concerns what each combination offers her, given her own constitution, experience, and previous sires. Following those evaluations through unequal paternal contributions and offspring performance would let us investigate whether female mate and sire selection favors genetically and microbially inherited capacities for constructing effective phenotypes under the circumstances encountered by the next generation.

The program therefore includes host genetics, immune regulation, epigenomics, and complementary measurements of gene activity, proteins, and chemistry. These are distinct approaches with connected explanatory jobs. Host epigenomics concerns regulation of the spider's own genome; community metagenomics concerns the genetic material and functional potential of its microbial associates. Their integration would allow us to investigate a developing host–microbe relationship rather than measure each component as though it existed independently.

Microbes as participants in the development of a male

The central hypothesis is not that a male develops a personality and then happens to acquire a microbiome. *It is that development of behavior, physiology, and microbial associations can become interdependent.* The male's inherited constitution and experience influence what microbial functions

he can recruit and sustain. Those functions, in turn, may support, extend, or constrain the capacities expressed in his reproductive behavior.

A useful association could help a male sustain demanding courtship, maintain metabolic functioning, limit disease damage, or preserve reproductive capacity later in life. The benefit need not be identical for every male. What supports one reproductive approach could be less useful, more costly, or unattainable for another. The relevant developmental outcome would be a fit among the individual's components, not possession of a universally superior microbial community.

A recent synthesis of 57 experimental studies provides a broader context for these questions. Schetini de Azevedo and colleagues (2026) document behavioral responses to microbiota manipulations, with substantial variation among investigations. *They also emphasize the concentration of evidence in laboratory model organisms and the need for longitudinal research that connects behavior, microbial functioning, and natural ecological circumstances.* In Sierra domes, I want to investigate how microbial functions participate in coordinating a male's characteristic behavior with his physical and physiological capacities as his life unfolds. Following individually known males allows us to connect their early circumstances and changing capacities with the ways they subsequently compete, court, and respond to females. Those histories give the microbial measurements a developing individual to help explain. Female responses, paternal contributions, and offspring performance would then allow us to investigate what the resulting male organizations accomplish reproductively, including whether a given male's package of traits reveals capacities that offspring can inherit and employ under their own circumstances.

Immune regulation offers one candidate means of constructing the fit among a male's behavior, physiological capacities, and microbial functioning. Defending the host does not necessarily mean excluding as many microbes as possible. Regulation can influence which associates establish, where they persist, and how abundant they become. In *Drosophila*, experimental changes to antimicrobial peptides and lysozymes altered the composition and abundance of gut bacteria (Marra et al., 2021). That supplies a concrete precedent for immune functions helping to organize an associated community rather than acting only as a wall against invasion.

My proposed extension is that this microbial management develops in coordination with the male's other capacities. The appropriate response might combine resistance, tolerance, containment, and permissiveness in different proportions. It could allow useful microbial activity while controlling its costs. The resulting association would depend both on the host's regulation and on the microbial opportunities available through prey, habitat, maternal transmission, or contact.

Host regulation is only part of the relationship. A male cannot recruit an organism that is unavailable, and the effects of an associate may change with nutrition or other circumstances. Henry and colleagues used reciprocal microbiome transplants in *Drosophila* populations with different dietary evolutionary histories and found that reproductive effects depended on the match among host history, microbial community, and diet (Henry et al., 2025). The result makes functional matching an experimentally approachable question. It does not imply that one microbiome improves every host under every condition.

The possibility of microbial assistance should not diminish our interest in the genetic basis of the male's own competence. Successfully recruiting and regulating microbial assistance could itself be a consequential host capacity. A male's body may obtain or accentuate functions from microbial associates that it could not perform as well alone. The question is how reliably he establishes the

right microbial relationships, how well he manages them, and whether offspring inherit capacities that help them establish useful relationships of their own.

This way of thinking broadens the role traditionally assigned to immunocompetence in sexual selection. Rather than treating the immune system mainly as a defense against parasites and pathogens that compromise performance, the hypothesis gives it a central role in supporting and potentiating adaptive developmental phenotypic plasticity. By helping recruit and regulate useful microbial functions, the immune system could participate in determining which combinations of capacities an individual can develop and sustain. It would help construct an effective phenotype as well as protect it.

Comizzoli and colleagues (2021) place these questions in the broader reproductive life of wild animals. Their review follows microbial influences from physiological regulation and reproductive signaling through mating, early development, and the later functioning of offspring. Microbial functions involved in reproduction can occur in the gut, on signaling surfaces, and in other parts of the body as well as in reproductive tissues. The authors also emphasize that these relationships can change with diet, captive conditions, and alterations of the natural environment. This gives their conservation argument a developmental dimension: successful reproduction may depend on preserving the environmental conditions under which useful host–microbe relationships are established and maintained. I find this perspective especially relevant to the Sierra dome program because the value of a given microbial association may depend on the individual acquiring it, the resources and challenges that individual encounters, and the stage of life at which the association becomes consequential. The spider work would connect those circumstances with known individuals' reproductive behavior and the subsequent performance of their offspring.

This conservation perspective also invites a question about the reproductive decisions through which particular inherited capacities are carried into the next generation. Female mate and sire selection may help maintain inherited capacities for establishing and regulating microbial relationships that support effective development. What females assess could include not only a male's ability to withstand infection, but also his ability to sustain useful associations and adjust their functioning as his circumstances change. If offspring inherit capacities that help them make comparable adjustments to their symbiont communities in their own lives, the resulting benefits to their survival and eventual reproduction could increase their mother's reproductive success. Selection could favor the female mate- and sire-selection responses responsible for those benefits. Improved population-level responsiveness to environmental perturbation could emerge as a consequence of these individual reproductive advantages; it is not the advantage invoked to explain the evolution of female choice.

Again, this would broaden the connection between immunocompetence and sexual selection. Hamilton and Zuk (1982) emphasized how continuing coevolution with parasites could maintain heritable fitness differences among males that are consequential to female choice and maintain its relevance. The present hypothesis asks whether the management of *beneficial* microbial functions also contributes to those differences, including through its potentiating effects on adaptive developmental plasticity. Continual reoptimization of an individual's symbiont community in the face of environmental insults and opportunities may make a major contribution to an individual's resilience, stabilizing health and strong expression or adaptive tuning of their genetically-based developmental program. If so, as seems plausible, then a female would not simply be selecting a presently successful male with good genes and microbes. She could be assessing capacities that help

offspring to dynamically construct effective combinations of their own physiology, behavior, and microbial assistance as development unfolds in a dynamic environment.

The conservation implication is that preserving animals, genetic diversity, and useful microbial associates may not fully preserve the processes that connect them. Opportunities for female mate and sire selection may also matter, particularly where those choices favor inherited capacities for responding to locally changing conditions. This possibility remains to be tested, but it gives the proposed work a significance beyond identifying microbes that improve an individual's health. It asks how female mate and sire selection, acting on a partner's capacity to recruit and regulate useful microbial functions, could help maintain the genetic basis for developmental responsiveness. Inherited capacities for immune regulation may be especially consequential because they could help offspring draw on microbial capabilities that extend what their own bodies can accomplish. The resulting contribution to resilience and future adaptive evolution could be substantial and greatly underestimated. Studies conducted (1) with simplified, ecologically unrepresentative, or debilitating microbial communities, or (2) under conditions that remove the ecological challenges and opportunities making these capacities valuable, may fail to reveal either their importance or their role in female choice.

Epigenetic regulation: how experience could become organized biology

Developmental experience can matter beyond its immediate behavioral effects. A difficult encounter, a nutritional history, or persistent physiological feedback could contribute to changes in how the individual subsequently regulates his functioning. Epigenetic processes are candidates for participating in that persistence and coordination. They would operate within interacting neural, endocrine, metabolic, and immune systems, not as a separate command center directing the rest of the animal.

A recent macaque study illustrates why this question is now approachable at a molecular level. Sadoughi and colleagues combined known life histories with DNA-methylation profiles from 2,485 samples across 14 tissues in 237 rhesus macaques. Different early-life adversities were associated with distinguishable signatures, while the effects of a particular adversity were partly shared across tissues. Age-related methylation differences were more tissue dependent, and adversity did not uniformly accelerate epigenetic age (Sadoughi et al., 2026).

For the Sierra dome program, the useful implication is not that macaques possess the reproductive behavioral genres proposed here. It is that a developmental history can leave a structured molecular pattern across different parts of an animal. We can ask whether recurring patterns of epigenomic regulation in males are associated with the coordinated behavioral and physiological combinations we observe, and whether those patterns contribute to the way the combinations develop.

The strongest version of the hypothesis concerns functional coordination, not merely molecular resemblance. A shared methylation pattern might record experience without causing the behavioral pattern of interest. Pairing epigenomic measurements with gene expression, immune functioning, microbial activity, and known performance histories would help distinguish a molecular record of events from regulation that helps organize the response to them. Nor does the hypothesis require every male within a genre to have an identical epigenomic profile. Similar functions may be supported by different individual arrangements.

There is already a spider-specific methodological precedent. Liu and colleagues combined whole-genome bisulfite sequencing with RNA sequencing in *Stegodyphus dumicola*, finding methylation

concentrated in genes and associations with the level and stability of gene expression (Liu et al., 2019). Those associations did not establish causation, but they show that paired epigenomic and expression measurements are feasible in a spider. They also provide a reason to investigate spider regulatory biology directly rather than assume that the meaning of methylation is identical to its meaning in mammals.

The particular connection I want to investigate is the regulation of microbial recruitment during the construction of a reproductive phenotype. Experience-responsive changes in host regulation could help shape an immune environment that supports microbial functions appropriate to a male's attainable approach. Those functions could then alter metabolism, physiological durability, and behavior, feeding back into the developing organization. Epigenetic studies would thus have a defined place in mating-system metagenomics: investigating how experience, host regulation, and microbial assistance become parts of the same individual life.

Inheriting responsiveness rather than copying an adult state

A father and his offspring do not have to end up with identical personalities, epigenetic profiles, or microbial communities for this developmental organization to have inherited consequences. The valuable contribution could be a capacity for responsiveness: the machinery that helps an individual adjust regulation, manage associates, and coordinate behavior with the possibilities available to its own body.

Genetic and epigenetic inheritance should be distinguished without imagining that they are unrelated. In *Nasonia*, allele-specific methylation patterns in hybrid offspring closely tracked those of the parental alleles, providing evidence for strong inherited control of methylation targeting (Wang, Werren, & Clark, 2016). That is different from demonstrating the transmission of an environmentally acquired mark. It shows why the genetic basis of epigenetic organization can matter even when inheritance of particular acquired states remains unresolved.

For the Sierra dome study, this makes the genetic basis of effective developmental responsiveness a possible target of female mate and sire choice. A male's performance and chemical credentials may reveal not merely what he has become, but something about how successfully his inherited regulatory capacities have helped him make use of his circumstances. By favoring him as a sire, a female could obtain genetic contributions to developmental regulation, including the epigenetic regulation of gene expression, that help her offspring make the best of the cards they themselves have been dealt. Those contributions could help offspring coordinate their behavior, physiology, immune functioning, and microbial associations as their own lives unfold. The proposed benefit is not that they become replicas of their father, but that they develop effective phenotypes suited to their own constitutions and circumstances. A son might consequently develop toward an individually attainable optimum within a different reproductive genre from his father, while a daughter could use related regulatory capacities to meet the different demands of her reproductive life.

Chemical credentials for an individually distinctive performance

The female observes a performance, not the complete organization that produced it. Courtship can provide substantial information about what a male is able and willing to sustain. Yet two males may reach a similar visible performance through different physiological arrangements, while two markedly different styles may express partly similar capacities. Chemical information could help the female interpret those distinctions.

A particularly suggestive precedent comes from Randy Thornhill's work on the Japanese scorpionfly, *Panorpa japonica*. Females preferred the pheromone of males with lower natural asymmetry in forewing length. Experimentally altering wing asymmetry by cutting did not redirect preference accordingly: attractiveness remained associated with the original asymmetry (Thornhill, 1992). The odor was therefore linked to an underlying difference among males rather than simply to their current wing geometry.

That finding helped motivate my interest in the chemical expression of developmental competence. A female need not inspect a morphological feature directly to receive chemical information associated with the processes that produced it. Behavior, morphology, and scent may provide different views of the same individual's functioning. The scorpionfly result does not identify a microbial mechanism, but it establishes a compelling reason to ask what physiological organization an attractive odor reveals.

Microbes may participate in producing that information. Gao and colleagues showed in two closely related flies that host-regulated amino-acid conditions influenced the mixture of bacteria-produced male sex-pheromone components; changing those conditions changed the chemistry and mating performance (Gao et al., 2023). The signal was a product of host physiology interacting with microbial metabolism. It was not merely a label showing that a particular bacterium was present.

This distinction is central to "olfactory credentials." Recruiting a useful microbial associate or an entire symbiotic community is one task; maintaining conditions under which it performs a useful function is another. Males carrying similar organisms could differ in the functions those organisms supply. Different assemblages might also support partly similar functions. Chemistry may be especially informative when its production or maintenance is causally tied to the capacities the female needs to assess.

Males never court and guard a non-virgin female for more than one period of daylight, so secondary mating encounters seldom last more than 10-12 hours. Under my hypothesis, microbially influenced odors or contact chemicals could corroborate, qualify, or contradict what a brief snapshot of the male's behavior suggests. The fact that a given male's precopulatory courtship—and, in even more disruptive weather or predatory encounters, his copulatory courtship—can be interrupted and delayed gives chemical assessments an even more important potential role. If a female captures a prey item during his visit, this can also delay courtship (Watson, 1993). A distinctive behavioral style might be revealed and backed by chemistry indicating an effectively functioning host–microbe combination. Another performance might look equally persuasive while its chemistry suggests a different physiological state. The information need not be a universal badge of superiority. It may help reveal what kind of male contribution to a female's brood is available and, mechanistically, how it is being sustained.

Related experiments make several parts of that question tangible. In a wolf spider, standardized male courtship videos elicited different female responses when accompanied by cuticular cues from infected rather than control males (Gilbert & Uetz, 2019). In thrips, endosymbiont associations were linked to changes in mating preferences and male chemical profiles, with consequences for compatibility (Tourani et al., 2026). Together with my published work on a volatile airborne female sex attractant in Sierra domes (Watson, 1986), which she places on the dome portion of her web as an emergency fertility measure *only* if she remains virgin 7-10 days after sexual maturation, these studies justify treating chemistry as a central source of reproductive information while testing its particular content and route in this species.

Chemical phenotypes also deserve developmental histories. Yukilevich and colleagues found that sexually informative cuticular-hydrocarbon profiles changed with maturation in *Drosophila* and used chemical-transfer experiments to test effects on male species discrimination (Yukilevich et al., 2026). This is a precedent for the developmental emergence of relevant chemistry, not a demonstration of female assessment of male microbiomes. For Sierra domes, the question is whether a male's changing chemical profile helps make the development of his reproductive capacities legible to females.

What the chemistry reveals and what it is worth

Two questions must remain separate: what the male's chemistry indicates, and what the indicated capacity is worth to the female receiving it. A cue can be reliable without being equally desirable to every female. One female might benefit greatly from the contribution it reveals; another might find it comparatively redundant or less compatible with her circumstances. Reliable information is not the same thing as universally valuable information.

This is particularly important when individuality persists within reproductive genres. A female is not choosing an abstract category. She encounters a male whose style has emerged from a particular history. Chemical signals and cues from the male could help identify capacities underlying that expression, while her own physiological state and previous matings affect their value. Her response might concern whether to mate, how his sperm is admitted or retained, or how much paternity he ultimately obtains.

The inherited-benefits question goes beyond detecting a good current performance. Does the chemistry identify capacities that offspring can inherit and express, including the capacity to recruit and regulate useful microbial functions? Or does it mainly reflect favorable exposure, present nutrition, or an association whose benefits are unlikely to be reconstructed by offspring? These alternatives give experimental work a purpose. They are not reasons to dismiss an effective host–microbe combination merely because microbes contribute to it.

No conscious classification or remembered ranking of males is required. Evolved sensory and physiological responses could integrate behavioral, vibratory, morphological, and chemical information in relation to the female's state. Similar overt acceptance could be followed by different sperm-use outcomes. The relevant evaluation may therefore be most apparent in sire contribution, even when observers see little difference in whether a female permits mating.

This brings the chemical investigation back to the original problem of unequal fertilization success. The goal is not only to show that a cocktail of informative male odors during courtship renders a female more sexually receptive and willing to allocate fertilizations to a male, although that is a worthy research goal. I wish to go further, asking whether chemistry adds information about a male, whether the effect of a male's scent on a female changes with the female's ecophysiological circumstances, and whether those female responses alter paternal contributions in ways that improve measurable fitness components of her offspring, like higher survival-enhancing early offspring growth rates, or their ability to recruit adaptive microbial symbionts to potentiate the best available developmental paths. Live-animal chemical measurements, including the recent honey-bee approach discussed in Section 5, make that chain of questions increasingly approachable.

A disease investigation with an existing molecular foundation

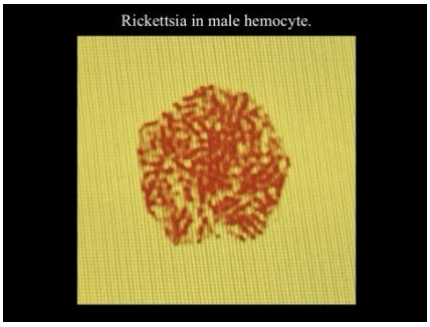
The microbial investigation also grows from conspicuous reproductive failure and premature mortality already repeatedly recorded in this population over many field seasons. My 1993 paper

and many subsequent years' records report exciting preliminary evidence consistent with mating-associated disease transmission in some years, alongside microscopic observations of organisms in developing eggs. Field and laboratory observations every breeding season have documented two particularly striking female reproductive-failure syndromes. These are repeated observations of severe disease symptoms unevenly distributed among individuals in the system, not a disease hypothesis inferred only from a sequencing result.

In the egg liquefaction syndrome, a female can initially look robust and beautifully gravid. She may begin building the bottom half of an egg sac, but then stall, become lethargic, and fail to oviposit. In the cases I have followed through to the end, the abdomen eventually ruptures, releasing liquefied egg material, and the female dies immediately. Most of my detailed observations of this progression have been made in captivity, after bringing known females into the laboratory shortly before their expected oviposition. The outward appearance of a large reproductive investment can therefore persist even as that investment is being lost.

Reproductive losses vary substantially among seasons. In my published study, the mean percentage of eggs that hatched was 51.76% across 133 broods from 1984–1985, compared with 72.56% across 43 broods in 1989 (Watson, 1993). Thus, in the earlier collection, the average proportion of eggs failing to hatch approached one-half. These percentages concern eggs laid by females that reached oviposition; they do not account for the additional reproductive losses among females that died before laying. The poor physical condition of inviable eggs and microscopic observations of rickettsia-like and virus-like organisms within developing oocytes implicated disease. The responsible agents and their transmission routes, however, remained unresolved, and the hatch percentages should not be interpreted as measurements of losses attributable to a single confirmed pathogen.

The hardened-egg syndrome often has a different sub-fertility outcome. A female may complete oviposition and produce a mixed clutch: some eggs are normal and they hatch into apparently healthy spiderlings, while others are hardened, gummy, and completely inviable. Successful laying is not equivalent to successful production of viable offspring. Photographs and repeated firsthand observations document these outcomes. Recent molecular work has begun to identify candidate pathogens (see below), but the causal assignment of particular organisms to these infertility and sub-fertility syndromes is mostly unresolved.



My observations of blood smears from male Sierra domes have revealed hemocytes fully loaded with organisms in some individuals. These organisms are vividly visualized using the rickettsia-specific differential staining techniques I learned from my UNM microbiology and rickettsiology mentor, Oswald Baca. The observations further suggest that male Sierra domes, too, are often battling a pathogenic organism of a rickettsial group.

The distinction between infertility and inviability matters. A dead or damaged egg need not be an egg that was never fertilized. Disease can interfere with egg development, the ability to lay, embryonic survival, or later emergence. Those outcomes have different implications for female reproductive success and for estimates of the contribution made by each sire. They also show why lifespan and outward condition are inadequate substitutes for sufficiently sustained reproductive functioning.

Molecular work has already begun to identify candidate agents. During two intensive summers of work in the FLBS molecular laboratory, I prepared many whole-spider DNA extracts and conducted candidate-directed bacterial screening, with extensive and generous training and guidance from FLBS scientists Logan Peoples, Matt Church, and Jim Elser, as well as consultation with other specialists, including investigators experienced with *Rickettsia*. The best tentative leads include organisms assigned to *Rickettsia* and *Spiroplasma*. Again, however, their causal roles in the two syndromes and their precise taxonomic status remain to be established.

A pooled metagenomic investigation undertaken in 2021 complemented the individual-spider DNA extractions and targeted bacterial screening described above. During July and August, I used Zymo's HostZERO Microbial DNA Kit to process pooled spider bodies collected from the FLBS study population. The procedure was intended to reduce the predominance of spider DNA and enrich the microbial component. I measured the recovered DNA using a Qubit fluorometer, combined the products of two preparations, and used DNA Clean & Concentrator-5 columns to concentrate the material for commercial sequencing. Logan Peoples provided extensive practical guidance during this work and then performed the computational analyses of the sequencing results. His contribution went well beyond reviewing a commercial report: he identified bacterial sequences within the mixed material and reconstructed substantial portions of their genomes.

Logan first examined 16S ribosomal RNA gene sequences, which provide recognizable genetic markers for distinguishing bacterial groups. His analysis retained seven bacterial sequences. The most strongly represented was assigned to Diplorickettsiaceae and appeared related to *Rickettsiella*, distinct from the separately identified *Rickettsia*. Other entries included *Wolbachia*, *Staphylococcus*, two provisionally *Spiroplasma*-related sequences, and a member of Micrococcaceae. He also distinguished these bacterial sequences from spider, fungal, and plant-associated sequences in the preparation. The findings agreed with our earlier targeted DNA tests, which had detected *Wolbachia*- and *Rickettsia*-related material. The pooled investigation therefore expanded an existing molecular inquiry, supplying additional organisms to investigate alongside the individual-spider screening already under way.

The more substantial achievement was recovering much of the genetic information belonging to several of these bacteria. Logan sorted assembled DNA fragments into groups judged to belong to the same organism, allowing draft genomes to be reconstructed from the mixed sample. Four reconstructions, assigned to *Staphylococcus*, Diplorickettsiaceae, *Rickettsia*, and *Wolbachia*, were estimated to contain roughly 82% to more than 99% of their respective genomes, with low estimates of contamination by other genomic material. A fifth reconstruction, associated with *Spiroplasma*, contained extensive duplication of genes used in the quality assessment. Logan suspected that two related genomes had been combined, consistent with the two *Spiroplasma*-related ribosomal sequences; resolving that mixture remains a task for further analysis. The important advance is that the investigation recovered extensive genetic information, not simply a short identifying sequence for each bacterial group.

In his preliminary assessment of 28 August 2021, Logan also considered the recovered bacteria likely to include previously undescribed species, opening another opportunity for comparative genomic investigation. Detailed examination of their genes could help identify functions worth testing in the spiders. The next task is to connect this genomic information more closely to individually characterized animals: determining which organisms occur in which spiders and relating those associations to physiological performance, courtship, reproductive health, and offspring outcomes. The technical foundation combines my earlier microbiological training with

rickettsiologist Oswald Baca at UNM and several mentors at Oxford's NERC Laboratory of Virology with the more recent FLBS-based work. This is a molecular investigation already begun, with retained laboratory records, specific bacterial leads, and substantial genomic information from which collaborators and successors can develop the next studies.

The work can advance from either end of the biological contrast. Severe pathology provides conspicuous failures to explain. Healthy, reproductively effective individuals provide opportunities to ask how functioning is maintained, including through beneficial or tolerable microbial associations. Both approaches belong within the same comprehensive mating system metagenomics investigation proposed here. Laboratory measurements would be linked to the behavioral histories and ecological circumstances of the spiders sampled, so that a microbial association can be interpreted in relation to the functioning of a known individual.

Immune-state-dependent compensatory sire choice

The female's own experience of disease could also alter what she benefits from obtaining in an additional sire. I hypothesize that difficulty sustaining reproductive health changes the relative value she assigns to particular paternal capacities. This is more specific than a general preference by unhealthy females for healthy males. It concerns whether a male offers a contribution that could reduce vulnerabilities made relevant by this particular female's physiological experience.

By physiological self-assessment I mean internal feedback capable of changing sensory or reproductive responses. Infection, costly immune activity, tissue damage, or disruption of egg development could supply such feedback. Her condition may contain information about how her inherited constitution is functioning under locally encountered challenges, even though it also reflects exposure, nutrition, and microbial circumstances. An evolved response need not diagnose each cause perfectly to use information that predicts useful differences among available sires.

A female struggling to maintain viable eggs could, under this hypothesis, place greater weight on evidence of effective defense or durable functioning in a secondary male. Courtship would show something of what he can perform; host- and microbially influenced chemistry could help reveal the organization supporting it. A useful inherited contribution might include the capacity to establish protective microbial functions, rather than simple inheritance of the exact organisms presently associated with the father.

The relevant capacities need not be narrowly effective against one pathogen strain that remains unchanged into the next generation. Recurrent local challenges, related strains, or functions useful across several challenges could make the female's current experience informative about offspring prospects. Species-typical requirements and population-specific microbial ecology would provide the broader context, while individual condition changes the weight of a contribution within it.

This argument retains the distinction between strong early performance and physiological durability. The behavioral-energetics records show why vigor at one moment should not be used as a complete measure of durable defense. The proposed value of several sources of information is that they may distinguish different physiological organizations capable of producing an impressive performance. What is useful depends on the female's vulnerabilities, her previous sires, and the lives her offspring will have to sustain.

The decisive prediction is an interaction between female state and male characteristics. The same characterized male, or the same combination of behavioral and chemical information, need not receive the same relative weighting from every female. A contribution could become more valuable

as her reproductive condition changes. This proposed input differs from the visible inferiority of a substituted champion in the first-mate experiment; it does not require her to erase first-mate precedence or assign individual fathers specifically to one offspring sex.

Direct protection and inherited benefits are different routes

The older NSF continuation proposal already considered whether mating and sperm-admission responses could protect the female directly from infection. Admitting an ejaculate to storage might create a different opportunity for a pathogen from leaving it elsewhere in the reproductive tract. The compensatory hypothesis adds a different question: whether an additional paternal contribution benefits offspring she remains capable of producing. A direct risk to her and an inherited benefit to offspring could favor different responses in the same encounter.

A third possibility would be a direct benefit from acquiring useful microbes or other substances during mating. That remains distinct from inheriting host capacities through a sire. Neither route should be assumed merely because a microorganism occurs in whole-spider material, and microbes have not yet been demonstrated in my collected semen samples. The accessible ejaculates described in Section 1 give us a way to directly investigate their microbial composition using a non-lethal method rather than guessing at the transmission route.

Rowe and colleagues (2020) discuss a particularly interesting form of the direct-acquisition possibility: a female might preferentially retain an ejaculate containing beneficial bacteria that are scarce or absent in her existing reproductive community. A male's value as a microbial contributor could therefore differ from his value as a sire. Acquiring a useful function from a male's semen, or from other material transferred during mating, might improve conditions for the female or for sperm already stored from other males, even if the donor ultimately obtains little or no paternity. The female might retain a useful microbial contribution while admitting or using little of that male's sperm. Mates obtaining little or no paternity have already been documented in Sierra domes; a microbial benefit from those encounters remains to be investigated.

This would provide an additional adaptive explanation for accepting a male who contributes little or nothing genetically. My earlier work identified enhanced female foraging success during copulatory courtship as a direct benefit of such encounters (Watson, 1993). Useful microbial acquisition could complement that nutritional advantage. If beneficial organisms or their products are transferred during the aspermic phase, a female might obtain a useful contribution before insemination, while continuing to benefit from the male's occupation with courtship rather than competing with her for prey. The timing and amount of any such transfer would determine whether an abbreviated encounter could supply that benefit. Earlier matings could also change the microbial circumstances in which a female encounters and behaviorally responds to later males. The identities, order, and timing of encounters would then help explain both the microbial relationships established and the paternal contributions eventually represented in her brood.

The beneficiary of a microbial function also matters. An association that supports a male's performance or protects his sperm could have different consequences in a female, while the microbe's own transmission interests need not coincide with either spider's reproductive interests. These possibilities make the host's ability to regulate microbial relationships especially consequential. I would want to distinguish what a male transfers, what that transfer does in a particular recipient, and what his genetic contribution subsequently accomplishes in offspring. Those effects may reinforce one another, but they need not do so.

The distinction between resistance and tolerance is also important when interpreting what a male's apparent health might offer a female. Resistance limits pathogen burden; tolerance limits the damage caused by a given burden (Ayres & Schneider, 2008). A male could remain an effective performer while carrying transmissible organisms, and another could look healthy because he experienced little exposure. The useful comparison links burden, host response, reproductive damage, and behavioral and chemical expression. It asks what apparent health means in the individual being evaluated.

Disease can also change a female's ability to assess males, not only the value of the contributions she would benefit from acquiring. In *Schizocosa ocreata*, uninfected females favored uninfected males, whereas infected females did not show the same discrimination in the reported experiment (Bauer-Nilsen et al., 2025). That is a useful alternative to automatic compensatory choosiness. A Sierra dome female experiencing informative physiological difficulty may respond differently from one already too impaired to assess or reproduce effectively.

The proposed compensatory response may therefore be most revealing before terminal deterioration. At that stage, her condition could supply information while she retains the capacity to evaluate males and produce viable offspring. Comparing functional females with females who are severely affected and handicapped in their mate and sire choosiness would help distinguish altered valuation from a loss of discrimination. I do not yet have a reliable behavioral indicator of an early disease-related change in a female's choosiness; Section 5 describes how independently characterized health and reproductive outcomes could be connected to her preceding responses.

The female-specific preference pattern thus gains a candidate physiological mechanism: her own functioning supplies information about what is needed, while a male's performance and chemical credentials supply information about what is available. Their interaction could change the composition and weighting of sires. The evolutionary test is whether females' selected male-specific paternity contributions improve the reproductive prospects of whole broods. That is where the proposed molecular mechanisms reconnect with the question that has motivated the mating-system study from the beginning.

5. A feasible program with useful results at each stage

The value of the Sierra dome spider as a model system warranting continued deep study does not depend on every proposed mechanism being correct. It lies in the ability to distinguish important alternatives through observations and experiments whose participants are known as individuals. A finding that rejects one part of the explanation would still teach us something consequential about development, chemical information, or female choice. The program is intended to leave successors a productive route into discovery, not a theory they must accept before beginning.

The central path runs from individual life histories and reproductive decisions to reproductive consequences. Repeated behavioral observations across contexts, especially with reference to the background behavioral energetics data sets, characterize the male's capacities and styles. Molecular and chemical work asks how those capacities develop and what information they make available to females. Female histories, parentage, and offspring studies then reveal how the information affects paternal contributions and what those contributions accomplish. Each connection is a scientific question in its own right.

These components need not be completed in just a few field seasons or by one investigator. Several complementary yet stand-alone research efforts can proceed in parallel, beginning where methods

and materials are already available. A focused behavioral, physiological, chemical, or developmental study can produce a substantial result while leaving a clear path to the next question. That is a practical advantage for students and successors seeking a coherent research experience or career-spanning research program of their own.

First: deepen individual histories

For a proposed 2027 field season, my priority is to continue and deepen the study of male personality and development that began in 2024 and continued in 2025. In those seasons, we followed individually known males from sexual maturation through adult life, repeatedly observing their courtship, fighting, and nighttime exploration. Most of the adult observations in 2027 would follow the same protocols, while increasing the number of repeated observations per male. This continuity would let us build on the existing records while obtaining a more detailed picture of how individual reproductive styles develop and change.

My plan is to undertake the main analysis of this developmental study after the 2027 season, when its records can be examined alongside those from 2024 and 2025. The earlier data remain unanalyzed, and we have relatively few repeated observations per male. Nevertheless, cumulative observation has left my assistants and me with a strong qualitative impression of males developing distinctive courtship and fighting styles, with no two males performing in quite the same way. The enlarged record should give us a better basis for distinguishing characteristic styles, adjustments to particular partners, and changes during a male's adult life.

The important extension in 2027 would be to begin each male's history earlier, approximately halfway to two-thirds through his penultimate instar—the last immature stage before sexual maturation—and then continue following him through adult life, unto death. The adult developmental investigation would continue; we would now be able to connect it to the young male's preceding life on his own web.

Competition before sexual maturity

Immature males are homebodies, much like females. They build and occupy their own foraging webs and ordinarily remain there unless another spider displaces them. But a homebody's life is hardly free of competition. This is a densely populated study site, and although there are plenty of places to put a web, there is substantial competition for the best ones. A male's competitive history begins well before his first opportunity to guard a female.

When I walk the study site before the breeding season begins, almost all the large webs in the most productive foraging sites are occupied by penultimate males. Immature females tend to occupy smaller webs lower to the ground. During the two instars preceding maturation, immature males outcompete females for these prime sites. A sizable part of the male population behaves as though the task is to become as large as possible without postponing maturity nearly as much as females do.

A productive web site has a particular geometry. Webs around chest height can intercept a good traffic of gnats, mosquitoes, and small mayflies, provided the surrounding vegetation leaves enough room for the insects to fly through. Yet open space alone is not enough. The spider needs appropriately positioned attachment points to support a strong web. Sierra domes are amazingly good at arranging their supporting silk lines to make use of those opportunities. The favorable combination is an unobstructed capture area without sacrificing the structural integrity of the web. Height, web size, vegetation, and the arrangement of support all belong in our account of what the male has obtained and must defend.

I expect a substantial positive relationship between the quality of these sites and the body masses of their occupants. Ferocity should matter too. Following the same males would let us investigate how possession of a good site, success against competitors, and subsequent mass gain fit together. A large male may obtain a productive site, while a productive site may help its occupant become larger. The sequence is part of the developmental story.

By the middle of his penultimate instar, a male has already had considerable experience of obtaining food, defending a resource, and operating within the possibilities and limitations of his changing body. I hypothesize that this supplies copious self-assessment information relevant to his eventual adult size, metabolic capacities, and ability to cope with disease and other challenges. Some of that information comes through encounters with conspecifics of both sexes. It could help organize a coordinated, multi-trait developmental trajectory before the male enters reproductive competition, with further tuning continuing as adult experience supplies new information.

Why early reproductive readiness matters

The seasonal timetable makes that preparation especially interesting. Male maturation is both earlier than female maturation—a pattern called protandry—and more tightly concentrated near the beginning of the breeding season. During two early seasons of my research, I monitored the proportions of males and females that were sexually mature on a daily basis. I am still astonished at the result. Pooling the two years, approximately half the male population was mature when only the first fraction of a percent of females had matured.

Seen at the population level, that timing hits a bullseye in targeting the first available newly mature females. The mechanism producing such close correspondence is unknown to me. Immature males are not patrolling the forest to estimate when females will mature; they are still on their own webs. Whatever developmental cues and regulatory processes produce the pattern, the resulting timing is consistent with a substantial reproductive advantage to individual males of being ready when those early opportunities appear.

A newly mature virgin female is fully receptive, and becoming her first mate brings the potentially large first-mate paternity advantage described earlier. There is also an early-season opportunity for males unlikely to prevail in the championship series. Once-mated females retain appreciable receptivity to secondary suitors, whereas rejection becomes much more frequent after a female's second mating (Watson, 1993). Early readiness can therefore matter to males with quite different bodies and competitive prospects, even though their best routes to reproductive success need not be the same.

My working hypothesis is that selection has favored males capable of arriving at this opening with their individual reproductive approach already reasonably well organized. They should be able to "hit the ground running," rather than begin every useful adjustment only after sexual maturation. This does not require every male to have the same optimal maturation date or the same behavioral starting point. A large, powerful male and a smaller male may have different opportunities to exploit. The common developmental problem is to make effective use of what each has become, while remaining responsive to what subsequent fights, courtship encounters, and the hazards of nomadic life reveal.

Females follow a different timetable. Their maturation is spread comparatively evenly across roughly six weeks after the breeding season begins. My interpretation is that selection gives females greater scope to delay the final molt while growing larger, because adult body size places an upper

limit on the number of eggs they can yolk up. Importantly, a female that matures later does not necessarily lose the competitive process by which she obtains her first mate and principal sire.

A late female can still elicit a championship

At this densely populated site, even late-maturing females generally encounter enough males for associative behavior to elicit a meaningful championship. I have not observed a female on my study site mature naturally without a preceding associative period or without a male present at maturation. A short associative period can limit the number of contenders, but an impression based only on daytime observations can be badly misleading. Most male movement between female webs occurs at night, and the rate at which males arrive can be surprisingly high.

There have been summers when I followed my marked females through the night. Sometimes two, three, or four males occupied a web at once. Keeping track of the dominant male could become a challenge in itself: by the time I had briefly removed him for marking and returned him, another male might already have arrived, perhaps within twenty minutes. This was not activity confined to a brief evening rush. It continued through the night. A female that becomes associative late in the day, or remains associative for only half a day or a day, may elicit far more competition than a daytime census would suggest.

The intensity can be remarkable enough that an associative female moves toward the periphery of the dome or even leaves it for the surrounding silk superstructure. I have seen this at night, when several males are competing in the dome, where their fighting occurs, usually near its center. My interpretation is that she is getting out of the way of potentially injurious combat, despite the greater exposure to predators outside the dome. These close-quarters encounters depend on tactile maneuvering rather than visual coordination, and several males simultaneously trying to engage one another can create considerable confusion.

Based on those observations, I regard five or more fights as a conservative expectation for many relatively short, late-season championships at this site. It is not a universal minimum: shorter series and solitary maturation remain possible, especially in less densely populated populations. The airborne attractant produced by females that remain unmated provides a means of obtaining a first mate when the usual route has failed (Watson, 1986). The important point is that late maturation generally does not dismantle the championship mechanism at FLBS. On average, a longer associative period and an earlier position in the breeding season bring more contenders, while web size and position also influence encounters. Searching males discover webs by contacting their silk and then investigate what is inside.

This contrast helps explain why male and female developmental schedules need not coincide. A female can often continue growing without entirely sacrificing competitive testing of her eventual first mate. A male benefits from being prepared to exploit the particular opportunities available when he matures. To understand that preparedness, we need to watch him while he is still growing, feeding, and defending a web of his own.

Recording the penultimate male’s life on his own web

Recognizing the relevant stage is straightforward with experience. Penultimate males are readily distinguished from adults and from antepenultimate males, which remain two molts from maturity. As the final molt approaches, the pedipalps darken from clear through gray to black and become more rounded. I would select males whose palpal tips are just beginning to show black coloration, using these familiar changes to estimate their position within the instar. We would weigh each male,

place a small paint mark on the tibia of a hind leg, and return him to his field web. I have used this marking technique thousands of times.

Frequent visits would connect the male's behavior with his web, its site, and his changing body mass. We would record web size, height above the ground, surrounding vegetation, and the openness and support that make the site more or less favorable for prey interception. Repeated weighing would establish the rate at which he gains mass. We would then obtain his actual maturation date and mass at that transition. My field observations indicate that larger males tend to mature earlier, but medium-sized and small males can also mature early. Those differences belong in the study rather than being averaged out of its developmental account.

A simple prey-presentation experiment would add a repeatable opportunity to observe the male's feeding behavior. We would drop a live, flight-impaired fly directly onto the dome and record how quickly and how boldly he responds. A small fly larger than a *Drosophila*, obtained with our fly trap, is suitable: sufficiently disabled to permit consistent placement, but alive and struggling on the silk. Keeping prey size, condition, and delivery as consistent as practicable would give us a manageable field comparison of the males' responses to a feeding opportunity.

We would also introduce penultimate intruders of known body mass onto the focal males' webs. Most would be similar in mass to the resident, with occasional larger intruders providing a different competitive challenge. The focal male would ordinarily be the resident. Once the intruder entered the dome with him, we would begin a thirty-minute observation and record the unfolding interaction. At the end, we would normally remove the introduced male. When an encounter begins late or becomes especially revealing, additional observation may be worthwhile, but that extension would remain distinguishable from the common thirty-minute record.

Preliminary observations in 2025 showed us what these encounters can look like. Almost all the penultimate males fought in the same manner as females: approach, slow circling, and then, if the encounter continued, pouncing and biting. The usual sequence lacked the adult's ritualized assessment stages. Nor did every introduction lead to fighting. Sometimes the resident or the intruder abandoned the web without a fight. Penultimate males do not readily tolerate sharing their webs, but there is more than one way for a contested occupation to end.

One male provided a tantalizing exception. He showed a rudimentary version of the adult Phase 1 display before he was sexually mature. I had not previously examined penultimate fighting systematically, and this glimpse of adult ritualization beginning to come online makes the proposed extension particularly intriguing. We can ask when such behavior first appears, how often it appears before maturation, and how it fits with the individual's subsequent adult fighting style.

The measurements would concern the manner of competing as well as the immediate outcome: approach, avoidance, persistence, readiness to attack, and the relative willingness of the two males to press the encounter. My earlier work found no resident advantage in adult fights, and the preliminary penultimate observations have not suggested an obvious one either. Nevertheless, residency would remain part of the record, to be examined alongside mass and behavior as the observations accumulate. Each staged contest would also become part of the focal male's developmental experience. Its place in his history matters to interpreting what he does next.

From homebody to nomad

The final molt provides a conspicuous event around which to organize these histories. We often see maturation itself; when we do not, frequent visits and the male's appearance generally allow me to

estimate its time to within a couple of hours. Importantly, a newly mature male does not immediately disappear into the forest. He remains on his web, usually for one or two days and sometimes three, while his new exoskeleton hardens and cures. He has stopped maintaining the web, but it still provides a place to remain during this vulnerable transition before he begins his nomadic reproductive life.

His changing coloration helps us read that transition. The cephalothorax goes from very pale gray to quite bright orange over roughly two or three days, more quickly in warm, dry weather. Together with increasing restlessness and movement within the web, that change tells me when departure is approaching. We would collect the male then and bring him into individual captive care for the repeated adult observations. The collection decision follows the animal's condition and behavior, not simply a fixed number of hours since his molt.

The paint mark is shed with the old exoskeleton, so continuity through maturation depends on following the male at his known web. I try to leave newly mature males in the field for as long as possible. Every extra hour in their natural circumstances is valuable, but by this point we have already invested substantial observation in each individual. There is a razor's edge here: collect too early and we unnecessarily curtail his field experience; wait too long and he vanishes with his history into the vegetation. In practice, reading the coloration and behavioral signs closely allows us to lose very few males at this transition.

After collection, individual indoor care would allow the adult histories to continue using the established approach described in Section 2. Courtship and fighting trials would still take place on natural webs in the field; captive care between observations makes it possible to find the same male again for his next encounter. We would continue to follow each male through adult life, record longevity, and preserve his body with its associated history when he dies.

An adult repertoire, performed in individual styles

The adult observations already reveal much more than differences in how vigorously males perform one standard routine. Watching their precopulatory strumming, I sometimes think of different rock stars and folk singers playing their guitars. There is a recognizable instrument and repertoire, yet the manner of performance can be distinctly individual. Much of the variation is continuous, while some males are conspicuous outliers. Repeatedly watching known males allows us to recognize these differences and ask how they persist, change, and respond to different partners.

In a staged adult fight, for example, an introduced male may dash toward the center of the dome or proceed slowly and cautiously. We record the time he takes to reach the center, who initiates the encounter, avoidance or delaying behavior, who initiates Phase 1 and Phase 2, and the intensity and course of escalation. Two males that both eventually engage in a fight can arrive there by quite different routes. A male's response to a larger opponent may also be characteristically different from his response to an evenly matched one. Keeping the opponent and the circumstances attached to each observation is essential to understanding the style.

The courtship comparisons concern encounters with non-virgin females. Precopulatory strumming is needed to elicit their receptivity; mature virgins are already receptive and do not present the same behavioral problem. A non-virgin female may respond to a suitor with a strong "no," including lunging or poking. Some males move away a little, circle, or maneuver to avoid the poke. Others stand their ground as though she were doing nothing. We have males who will not take no for an answer, and others who stop courting and turn their attention to foraging.

That change of activity is biologically consequential. Adult males no longer build their own foraging webs, so a female's web can offer food as well as a mating opportunity. Persistence, withdrawal, repositioning, and a switch to feeding are therefore responses within a real reproductive and energetic predicament. Repeated encounters with different females would let us examine whether a male has a recognizable way of handling rejection, how he adjusts to the female in front of him, and whether those responses become reorganized during his adult life.

Laser vibrometry would add much finer measurements of the strumming itself and of the female's signaling responses. I would use it for repeated observations of known males with different non-virgin females, in suitable indoor space near the field site. The reason for considering a substantial personal investment in a system is to obtain detailed performances from individuals whose lives we are already following, rather than accumulate recordings from unfamiliar males. The successful 2025 pilot and the proposed instrumentation are described in the following vibrometry subsection.

Nighttime exploration would continue to be measured only after maturation. Penultimate males are web occupants, not between-web explorers; their movement within a web should not be confused with the adult's nomadic search. Repeated adult observations in the infrared-monitored enclosure would allow us to ask whether earlier growth, web defense, and prey-capture behavior help predict the exploratory styles that appear after this transition. We do not yet know whether the exploration records will reveal developmental patterns comparable to those we suspect in courtship and fighting.

What the longer histories would let us ask

The enlarged study would connect activities that occur on opposite sides of a major change in the male's life. Does a fierce defender of a penultimate foraging web become an equally persistent adult competitor, or does his approach change as adult ritualization and different opponents alter what works? Does a male's tendency to approach a challenge cautiously recur in other contexts? How do mass gain and maturation timing relate to his early adult responses, and how do those responses change with experience and age? The same male supplies the continuity, while the changing challenges give development something to explain.

The broader aim is to follow how exploration, competition, and courtship become coordinated with a male's physical and physiological capacities, health, and eventual longevity. The behavioral record would provide the foundation for the physiological and microbial investigations described elsewhere in this prospectus. It would also allow us to examine reproductive genres as recurring combinations emerging within individual lives, while retaining the distinctive details of each male's performance. We need to know both what related males have in common and how each makes use of his own circumstances.

A male defending a productive web, a newly mature male waiting for his exoskeleton to harden, and an adult responding to a female's vigorous rejection are successive chapters in one life. Following those chapters is how we can move from recognizing that males have different styles to understanding how those styles develop, what they enable their owners to accomplish, and what females make of them.

An existing publication opportunity can proceed alongside fieldwork

The developmental histories collected in 2024 and 2025, and those proposed for 2027, are not emerging in a physiological vacuum. My earlier behavioral-energetics investigations generated a substantial body of physiological and behavioral records on individually known males. They connect

the energetic demands of fighting and aspermic copulatory courtship to continuously observed performances, and the repeated copulatory-courtship measurements reveal changes in metabolic performance through adult life. Respirometry exposed individual differences and population-average costs of species-typical, 'stereotypical' behaviors—including the average costs of Phase 1 versus Phase 2 ritualized fighting—with detail that even the best behavioral observations could not supply. Similar visible performances by two males, or by the same male at different ages, can entail quite different metabolic expenditures. These investigations approach individual reproductive differences from a complementary direction: what the male's body must expend, accomplish, and sustain.

A physiological record tied to what the male accomplishes

The principal fighting-energetics findings are published, whereas the much larger copulatory-courtship investigation remains far from completely published. Its raw respirometry files and accompanying behavioral and individual records are comprehensively preserved and available for analysis. They concern captive, individually known males whose records include body mass, longevity, and relevant collection circumstances—for example, a first mate captured after his championship, a secondary mate, or a randomly collected male. The repeated measurements and preliminary analyses summarized in Section 2 are parts of this substantial physiological investigation, not substitutes for examining the larger collection.

A common behavioral measure connects those records with work before and after the respirometry years. For copulatory courtship in either the field or the laboratory, I record 'hit rates': three-minute counts of successful genital couplings, or hits, and unsuccessful attempts, or flubs. I take these counts at temporally separated points in a performance, generally beginning after the male has had roughly fifteen to thirty minutes to warm up, then sampling again to follow his rise in performance and later during its sustained portion. The male is repeatedly operating elaborate palpal machinery, and counting attempted movements without distinguishing successful couplings would miss an important part of what he accomplishes.

Respirometry adds the physiological history behind that visible sequence. Some males sustain a comparatively smooth metabolic plateau; in others, the supposed plateau looks more like rough terrain. A male may interrupt copulatory courtship to groom his pedipalps, with a sharp fall in metabolic rate during the interruption. Flubs, involving incomplete inflation of the palpal hydraulic apparatus, also need to be distinguished from fully executed couplings. The paired observations allow us to ask what the male was doing when the trace changed, rather than treating every irregularity as the same physiological phenomenon. They also preserve questions about the expenditure associated with successful coupling and its duration, to be addressed at the temporal resolution the respirometry records permit.

What further analysis can contribute

There are consequently two connected timescales for further analysis: change within a courtship, from the initial rise through sustained performance and interruptions, and change between successive courtships as the male ages. A focused paper could examine individual combinations of maximum metabolic output, speed of mobilizing that output, energetic efficiency, and sustained performance. Another could examine their relationship to subsequent decline and longevity. The intriguing preliminary finding of strong early metabolic performance followed by faster decline

gives the latter question an empirical starting point; the larger archive provides an opportunity to investigate its generality, not a reason to announce it as established for every male studied.

These comparisons could enrich our interpretation of reproductive genres. Do males that perform copulatory courtship at a high rate for a shorter time differ in their combination of maximum output, energetic efficiency, and capacity for sustained performance from males that maintain a more modest rate for longer? Which combinations accompany different ways of competing, and which remain effective later in life? Those are questions about the physiological organization supporting a reproductive approach, not an assumption that one kind of male is simply a sprinter and another an endurance athlete.

The connection to the personality study is direct. Its known males provide behavioral observations of fighting and, when mating proceeds, copulatory courtship, including the familiar hit-and-flub measurements. The older physiological work therefore informs the interpretation of activities we continue to observe, even though it does not measure the current males' individual expenditures. It does not establish the energetic costs of precopulatory web-strumming, which is a different performance. I am not proposing to restart respirometry as part of my own immediate field program; the retained data can contribute now, while a successor could pursue additional physiological measurements as a separate investigation.

The archived NSF continuation proposal supplies a useful methods map. It documents DATACAN V processing of the traces, baseline and temperature adjustments, mass standardization, and behavioral annotations synchronized with gas-exchange measurements. Using the documented definitions of maximum output, relative energetic efficiency, and related variables, a collaborator can extract measurements with their biological meaning and individual histories intact (Watson, archived NSF continuation proposal, PDF pp. 12, 15). The task is further extraction, analysis, and publication of retained research, not reconstruction of lost observations. The historical paternity records, by contrast, have already yielded their principal analyses; their continued availability supports verification, interpretation, and comparison with new investigations.

This publication work can proceed alongside new field observations, to the extent that collaborators, their time, and support for their contributions become available. It is not tied to a promise that I will personally complete every analysis during the next field season. Nor must it await demonstration of reproductive genres or completion of the microbial investigation. Each bounded analysis can make the existing physiological foundation more accessible while helping an investigator identify the next question worth asking of a living male.

Laser vibrometry adds enormous detail to an observable interaction

Respirometry taught me how much a familiar-looking performance can conceal. Laser vibrometry offers a comparable advance in resolving the fine structure of precopulatory courtship and the female's replies. Many strumming movements are too fast, and their bouts too brief, for even a practiced observer to do much more than recognize the behavior and give a relatively crude assessment of its frequency and intensity. A detailed vibration trace lets us examine how the performance is organized, rather than merely count that it occurred.

The 2025 proof of concept

In 2025, Polytec generously loaned us a vibrometer and heavy-duty tripod for two or three weeks. Most of that interval went into getting the supplied software working on my Lenovo and developing the technique for aiming the laser and obtaining a good reflected signal. Only a few days remained

for recording spiders. We deliberately selected a small number of males whom we already knew from the field personality study to be avid precopulatory courters. The purpose was to establish that useful recordings could be obtained, not to sample male diversity. We did not obtain repeated recordings of individual males with different females. Once the arrangement worked, however, I was surprised by how well it worked, and by how efficiently detailed records could be collected.

We staged encounters in cages where females had constructed good webs. The laser was directed at an extremely lightweight square of foil, less than one square centimeter in area, suspended in the dome and positioned to reflect the beam back to the instrument. The software displayed the vibrations against time in real time on the computer. Often my assistant watched the spiders continuously and called out their actions while I watched the trace develop. The files remain saved by male identity, and some have accompanying handwritten annotations that identify the file and the precise times of observed behaviors. Those notes preserve the association between particular portions of a trace and the animals' actions.

The pilot revealed recognizable patterns associated with the male's typical four-legged courtship strumming, abdominal twitching, and pedipalp wiggling. The female's two-legged rejection strumming, silk pulling, and a few instances of skipping also produced distinguishable patterns. This matters because the interaction is not a male recital delivered to a passive audience. The female replies, rejects, pauses, or becomes receptive; the male may persist, reposition himself, or change what he is doing. When he abandons precopulatory courtship and turns to foraging, the courtship-associated abdominal twitching stops and he moves to dome center and becomes still. That familiar foraging posture is a change of activity, not simply a quieter version of courtship.

From recognizable displays to individual styles

The most exciting opportunity is to resolve the different 'guitar-playing styles' of known males. We could quantify the structure of strumming bouts, their rhythm and persistence, the arrangement of pauses, and the changes that follow particular female responses. The pilot established that familiar behaviors could be connected to detailed vibration records. Repeatable individual styles remain the subject of the proposed investigation. Repeated recordings with different non-virgin females would let us ask which features recur in a male's performances, which are characteristic adjustments to his partner, and which change as he develops and ages. The identity and condition of the female, the web, and the recording arrangement would remain attached to each trace.

One specific hypothesis is that males whose developing reproductive approach places greater emphasis on obtaining secondary matings will develop more elaborate or persistent precopulatory strumming than males whose strengths lie principally in championship fighting. Such a relationship would connect a fine-grained performance to the wider organization of the male's reproductive life. It need not divide the population into two mutually exclusive types: a championship winner can also pursue secondary matings, and individual males may combine strengths in different ways. The question is whether characteristic signaling styles become associated with particular combinations of competitive capacities and reproductive opportunities.

Laser vibrometry has resolved elaborate courtship in other spiders (Sivalinghem & Mason, 2021). In a leafhopper, Briggs, Espindola, and Krugner identified distinct male mating, male rivalry, and female signals, with a rival's presence redirecting male signaling toward competition (Briggs et al., 2026). These studies suggest useful comparisons of signaling across circumstances, rather than a repertoire to assume for Sierra domes. Our own pilot supplies the direct evidence that the method is

feasible here, and the ongoing personality study supplies individually familiar performers whose responses to different circumstances can be followed.

For the proposed 2027 studies, I plan to buy or rent a vibrometry system with personal funds. The next step would pair repeated indoor recordings with the expanded field observations of those same males, not replace natural encounters with an instrument-only study. An additional trained assistant would allow more recordings and repeated observations while the established field protocols continue. The number of encounters must remain matched to the people available to observe and document them properly.

For the personality study, this adds a much finer account of how each male performs and responds. For mating-system metagenomics, it supplies better-characterized behavioral phenotypes to relate to host physiology, microbial associations, emitted chemistry, and female evaluation. Respirometry measures expenditure; vibrometry resolves vibration patterns. Neither substitutes for knowing what the animals are doing. Together with that knowledge, they let us ask how an individually distinctive performance is produced, what sustains it, and what the female learns from it.

Measuring chemistry while the individual is still alive

The next connection is to the chemistry of those same living individuals. The longitudinal program needs measurements that can be linked to a male's life without requiring that life to end at the first molecular sample. Preserved endpoint specimens remain valuable, but they cannot reveal the full sequence through which a chemical or microbial state arose. An individual whose behavior and physiology are changing is more informative when at least some associated chemistry can be measured along the way.

Fido and colleagues provide a particularly relevant methodological precedent. Using secondary electrospray ionization–high-resolution mass spectrometry, they measured compounds emitted into the air surrounding individual living honey bees. Chemical profiles differed with controlled gut-microbial colonization, and infection-associated profiles could also be distinguished. The investigators then sacrificed the bees for accompanying assays; the study did not demonstrate repeated longitudinal sampling of the same surviving individuals (Fido et al., 2026).

The opportunity for Sierra domes is nevertheless substantial. A sufficiently sensitive, non-destructive measurement of emitted chemistry could allow a male to return to his behavioral study after sampling. His chemical history could then be considered alongside the development of exploration, courtship, fighting style, and eventual longevity. That extension would need validation in a much smaller animal, including whether the collection conditions alter subsequent behavior. It is a proposed application of the live-insect approach, not an already established Sierra dome method.

This gives the chemical investigation a direction beyond asking which odors separate two groups of preserved animals. Does the profile change as a male's reproductive approach develops? Does it change when his physiological performance deteriorates? Does a chemical change precede, accompany, or follow a change that an observer can recognize? Each comparison could help distinguish chemistry that reflects the construction of a capacity from chemistry that mainly records its loss.

Analytical detection alone would not tell us that a female perceives or uses the compounds. It would identify candidates for the behavioral experiments described below. The opportunity is to connect

measurement of a living male's chemistry to the next natural encounter, and eventually to its reproductive consequences, rather than stop at a chemical fingerprint.

Secretions and excreta: repeated samples from known males

Airborne chemistry is not our only opportunity to obtain material while a male continues his behavioral history. Fresh ejaculates, the secretion used in palpal grooming, and excreted droplets are externally obtainable samples from a known individual. Their accessibility grows out of the same advantage that makes the behavior so tractable: we can see what the spider is doing on his web, recognize the approaching event, and be ready to collect its product.

The semen-collection method described in Section 1 can be elicited from a selected adult male at a chosen point in his study. A male followed through his penultimate and adult life can be introduced to a mature virgin female on an accessible web. Her receptivity allows the sequence to proceed without precopulatory strumming, but the male still performs the full aspermic copulatory courtship before semen loading and insemination. His two fresh ejaculate deposits provide distinguishable sampling opportunities within that sequence. Their composition can then be investigated in relation to his age, prior encounters, copulatory performance, and eventual longevity.

Collection of semen is established; validated analyses of these very small samples are the next task. I have not yet demonstrated microbes in the droplets. The samples nevertheless remain useful for investigating other seminal constituents and possible relationships to host–microbe functioning elsewhere in the male. Collection order and the quantity removed also belong in the history: removing an ejaculate changes what is available for the ensuing insemination, and the preceding copulatory courtship is itself an energetically demanding experience.

Palpal grooming supplies a different material. While preparing and maintaining his copulatory machinery, a male accumulates a tiny, visible mucous droplet at his mouthparts and uses it in grooming and lubrication of the pedipalps. It is exclusively associated with preparation for and performance of copulatory courtship and the insemination phase of mating. The droplet can be collected and examined; it is not semen. Its chemistry and any microbial contents offer questions about a substance used directly on the structures whose repeated successful operation we measure as hits and flubs. Whether microbial associates occur in it, and whether its properties relate to copulatory performance, remain questions for analysis rather than conclusions drawn from its appearance.

The contents of the palpi during copulatory courtship offer a separate question. My early examinations established that they contain no sperm during this phase, but their other contents and any material transferred to the female have not been investigated. Courtship intromissions commonly last only one to three seconds when a male is performing effectively, whereas insemination intromissions are much longer, often around forty-five seconds. This difference may help identify how the two phases function, but it does not establish that the hundreds of shorter copulatory courtship contacts transfer nothing. Comparisons among palpal contents, external grooming material, and female reproductive material before and after aspermic courtship could ask whether compounds or microorganisms are transferred before sperm arrive, and whether any such transfer changes the subsequent environment for sperm. The observable separation of courtship, semen loading, and insemination makes the timing of these possible effects unusually accessible.

The female's pursuit of prey introduces a further potential conflict during these encounters. I have repeatedly observed females leave the copulatory position to capture prey, sometimes while a male's palp remains attached, with resulting risk of palpal injury. Prey capture usually ends the encounter; occasional resumption after consumption of a small prey item occurs, but appears uncommon in my observations. The retained records have not yet been analyzed for that pattern. I hypothesize that the tendency of secondary mates to restrict copulatory courtship and insemination to one pedipalp helps preserve the other for subsequent encounters. This could reduce the risk of losing all effective mating capacity during an encounter offering relatively little prospective paternity.

Excreted droplets offer a still more routine opportunity. A spider usually hangs horizontally beneath the dome. Before releasing a droplet, he moves into a vertical body posture, often without changing his location at dome center. Once one knows that posture, the impending droplet is easy to anticipate and its landing place reasonably predictable. We can intercept it on a glass slide and transfer the material into ethanol. Collection can be repeated while the male remains part of the behavioral study, without puncturing him or requiring a mating encounter. Feeding history, collection time, body mass, and recent activity would accompany each sample, so that changes in the material could be related to changes in the same male's life.

Samples could be collected on a standardized schedule and around selected events, including maturation and characterized reproductive encounters. Material collected on slides could be transferred into molecular-grade ethanol and chilled, or retained on frozen slides, with handling chosen for the intended measurements. Paired samples would allow us to ask which microbial organisms, genes, or products detected in excreta also occur in ejaculates or grooming secretions, and which remain specific to the sampled material. Any useful correspondence would make routine excreta sampling more informative; an absence of correspondence would also help locate the relevant processes. Their value as indicators of reproductive tissues or ejaculates must of course be tested, not assumed.

The analytical objective is to learn what each material reveals. An ejaculate, a grooming secretion, and an excreted droplet are not interchangeable views of the animal's physiology or microbial associations. Chemical or microbial differences in them would need to be related to independently characterized performance and condition before being used as indicators of either. Appropriate slide, fluid, web, and extraction controls would distinguish material associated with the spider from collection and reagent background. Sample handling would be matched to the intended analysis; a demonstrated means of obtaining a droplet is a starting point for developing the assay.

Hemolymph sampling is also possible, but it is trickier and more intrusive. A sample that leaves a male alive could still alter his subsequent performance and apparent personality. The effects of collection would therefore need evaluation before repeated blood sampling became part of a longitudinal protocol. The more accessible secretions and excreta allow the investigation to begin without making that procedure a requirement. Endpoint bodies remain complementary resources; the important opportunity is to obtain useful measurements before the individual's behavioral history has ended.

From molecular identities to functions and developmental organization

The pooled metagenomic results, individual-spider screening, existing pathological observations, and recent preserved specimens provide starting points for more targeted investigation. Further individual-linked work would ask which organisms occur in which spiders, at what burdens and

locations, and how those relationships differ among healthy, deteriorating, and reproductively impaired individuals. It would connect these existing molecular results more closely to biological variation among males and females.

I have a confirmed recent collection of males and females stored individually in molecular-grade ethanol and frozen at approximately −25°C. Its associated records can be examined to establish which behavioral histories and reproductive outcomes are represented. This collection should not be conflated with older bodies preserved during the respirometry years, whose present availability is unconfirmed. The original behavioral and physiological records remain available regardless. New cohorts would deliberately connect specimens and samples to the individual histories and outcomes needed for the particular molecular comparison.

Metagenomic sequencing can identify organisms and potential functions. Measurements of host and microbial gene activity, proteins, and metabolites can then ask which functions are operating in a particular individual. These approaches should be chosen because they help explain a performance or reproductive outcome, not because collecting more molecular categories is an end in itself. The behavioral record gives the measurements a set of questions to answer.

Epigenomic work would add a developmental dimension to that comparison. One useful design would link known early circumstances and repeated behavioral observations to molecular samples collected at defined developmental stages. Comparisons among appropriate tissues, or among matched cohorts sampled at different stages, could ask whether neural, metabolic, and immune regulation change in coordinated ways. Not every tissue can be sampled repeatedly from a living spider, so longitudinal behavioral records and carefully chosen endpoint measurements would remain complementary.

The macaque and spider studies discussed in Section 4 support this direction without prescribing a single assay for Sierra domes. DNA methylation is one component of epigenetic regulation, and a broad shared signature is not automatically the signature responsible for behavior. Combining it with gene-expression and physiological measurements would help determine what the molecular differences mean. Age, tissue composition, developmental stage, and health would be part of that interpretation, rather than compressing them into one presumed measure of biological age.

The decisive developmental comparison is whether host regulation and microbial activity accompany the emergence or maintenance of a reproductive combination. A useful microbial function might become established as a male's behavior and physiology come into closer alignment; alternatively, the association might appear only after deterioration. Those possibilities require different explanations. Measurements during life and at selected stages would give us a better basis for distinguishing them than terminal specimens alone.

Testing what chemical information adds

Chemical experiments should ask more than whether an extract or odor is attractive. Candidate airborne and contact-associated compounds could be characterized and presented with a controlled or well-characterized courtship performance. The question would be whether the added chemistry changes the female's evaluation, and whether it does so differently according to her own condition and reproductive history. The behavioral and chemical components would thereby become experimentally separable without losing their normal relationship.

The comparative studies provide useful approaches rather than templates to copy wholesale. Chemical transfer and manipulated cue presentation can test whether a profile changes a response,

as illustrated by Yukilevich and colleagues and by the wolf-spider work discussed above. The Sierra dome question is then what the active chemistry reveals about a characterized male, rather than assuming that every effective chemical constituent conveys the same sort of information.

Complementary manipulations of a microbial association could ask whether it changes the male's performance, the associated chemistry, or both. An effect on nutrition or health might itself be an important microbial function; it is not automatically an alternative to one. The experimental distinction is between effects of changing the association and effects of the treatment that occur independently of that change. Pairing those comparisons with controlled chemical presentation would then help separate a microbial contribution to constructing the male's phenotype from a chemical contribution to the female's assessment. Demonstrating both would be particularly informative, but a result at either level could stand as a useful contribution.

The female's response must also be followed far enough to identify what changed. An odor might affect initial acceptance without changing subsequent paternity, or influence sire contribution even when she accepts mating equally readily. The biology of this system makes that distinction unusually accessible, because courtship, semen loading, and insemination are separated in time and can be observed directly.

Connecting outward performance to hidden reproductive outcomes

A renewed parentage study with validated, more precise molecular markers could revisit the earlier sperm-use findings while connecting paternity to detailed male and female histories. The opportunity is not simply to repeat the old work with a newer assay. It is to characterize the male more fully and to retain the female's state, prior mates, and responses as part of the explanation of unequal fertilization success.

Modern reproductive-tract imaging and functional observations could test the candidate sperm-admission mechanism. The anatomical observation provides a place to investigate; it does not establish that one valve explains every paternity pattern. Comparisons among related species could also clarify how male genital reach and female tract architecture affect placement and storage. Those anatomical questions would complement, rather than substitute for, the behavioral and parentage records.

The successive outcomes must be connected without collapsing them. Delivery of an ejaculate, admission to storage, retention, fertilization, and survival of the resulting offspring are different events. A female could accept a male yet admit little of his contribution, or store sperm in proportions that differ from the shares subsequently used. Following those transitions is how a chemical or behavioral response can be connected to actual sire success.

Parentage at different developmental points is especially important in a population with reproductive disease. A sire might become more common among surviving offspring because his offspring survive better, rather than because his sperm initially fertilized more eggs. An apparently normal egg sac can also conceal mixed viability. Newly laid eggs, developing embryos, and surviving offspring therefore answer related but different questions about the same reproductive outcome.

This does not assign every difference to female control in advance. Ejaculate composition and sperm competition also belong in the investigation. The purpose is to identify the mechanisms producing the shares, including where the female's responses enter the sequence, and then evaluate their consequences. A larger fraction of fertilization success explained would be valuable because the added explanatory power would be connected to a biological process.

Rowe and colleagues (2020) identify several microbial and immunological interactions that could help explain those reproductive transitions. Microbes or their products may affect sperm directly; seminal constituents may protect sperm; and a female immune response directed at microbes may also alter sperm survival or functioning. Interactions between an incoming ejaculate and the female's existing microbial community could make the outcome depend on the particular partners and their preceding histories. Such effects could participate in adaptive female sperm use, arise through more general defenses, or reflect competition and damage whose consequences differ among the participants. The Sierra dome system gives us an opportunity to begin investigating this sequence with a known male's behavior observed and his fresh ejaculate accessible before insemination. As analytical methods for these tiny samples become workable, their properties could be connected to the recipient's microbial and physiological condition, sperm storage, fertilization, and components of offspring fitness. That would put biological mechanisms behind the paternity differences while retaining the question of what those differences accomplish for the female's brood.

From male reproductive genres to nonrandom sperm use

The preceding questions follow a male's outward performance into his reproductive contribution. I want to take that connection one level further, without losing the integrated individual whose performance the female has assessed. Selecting a particular father does not necessarily settle which of his possible haploid genetic contributions will enter the brood. I want to ask whether the host–microbe relationships that participate in constructing his reproductive phenotype also help shape the genetic composition of the sperm he makes available, and whether the female's reproductive environment further changes which of those sperm succeed. This would connect the developmental organization described earlier with the actual paternal contributions represented in offspring.

There are comparative reasons to take selection within an ejaculate seriously. In mammals, some gene products are incompletely shared among developing spermatids, allowing differences among sperm to reflect their haploid genomes (Bhutani et al., 2021). Experimental work in zebrafish has connected selection among sperm from the same male with differences in offspring performance, while accompanying analyses identified genetic differences among sperm subpopulations (Alavioon et al., 2017). In sticklebacks, Lenz and colleagues (2018) reported evidence of fertilization biased toward particular combinations of maternal and paternal major histocompatibility complex (MHC) haplotypes, including discrimination between alternative haplotypes supplied by the same male. These findings provide precedents for investigating nonrandom genetic contributions within sires; they do not establish microbial participation in such discrimination. The MHC example concerns vertebrate immune genes, not an immune mechanism to presume in spiders.

In a developing male, microbial products and the physiological conditions maintained through host regulation could influence which sperm survive, mature successfully, or remain capable of contributing to an ejaculate. If sperm bearing different haploid genotypes differ in their responses to those conditions, the resulting contribution could be a nonrandom sample of the male's potential meiotic products. The same microbial associates involved in constructing his behavioral and physiological capacities might participate, or their effects might be mediated through host processes or microbial functions elsewhere in his body. The important connection would be between developmental organization and genetic representation, not necessarily the presence of an identical community in every tissue. Such filtering would not require microbes to alter recombination itself; it could change the representation of the genetic combinations that meiosis has already produced.

This would not require an ejaculate to become genetically uniform. A useful outcome might be enrichment of several complementary contributions or reduced representation of particular combinations that function poorly under relevant conditions. Nor would a sperm transmit the male's entire integrated phenotype: it carries a haploid genetic complement, not a copy of his complete diploid constitution, developmental history, or microbial community. The question is whether his organization predicts a functionally structured distribution of potential contributions, including capacities that help offspring develop appropriately under circumstances different from his own.

A male's distinctive behavior and chemistry could then provide information about the distribution of contributions he offers, rather than simply indicate whether he is vigorous or healthy. Selection could favor that correspondence when females benefit from recognizing those contributions and males benefit from their successful use. The connection might arise because some of the same physiological, regulatory, and microbial processes support both the assessed phenotype and the production or maintenance of particular sperm. Whether the phenotype predicts anything this specific about the available sperm remains an empirical question. The interests involved need not always agree: a sperm-level transmission advantage could favor a selfish genetic element rather than improve the reproductive prospects of either parent or their offspring (Bhutani et al., 2021). The consequences would therefore determine when a reliable correspondence is also a useful one for the female.

The female could introduce further discrimination after receiving the male's contribution. Conditions affecting admission to storage, survival during storage, activation, release, and fertilization might favor different sperm according to their characteristics and their compatibility with the female or her eggs. Microbial activity and host immune regulation are candidate participants in those conditions. Earlier encounters, potentially including material transferred during aspermic copulatory courtship, could also alter the environment confronting later ejaculates. The question is whether these processes distinguish genetically consequential differences among sperm within a sire, as well as changing the relative representation of different sires. The very brief interval over which a female lays her eggs need not be the entire opportunity for discrimination: some filtering could occur during the much longer period of sperm storage. The precise site and timing of fertilization in this species would remain questions to establish, rather than assumptions about how the final matching occurs.

One particularly important possible benefit would be reducing intralocus sexual conflict between the inherited requirements of sons and daughters. Some paternal contributions that support successful male competition may have different consequences when expressed in females whose reproductive success depends on sustained functioning through egg production and oviposition. Following identification of the Sierra dome's sex-determination mechanism, I would want to ask whether particular paternal genetic contributions are represented differently in sons and daughters in ways that improve their respective reproductive prospects. The relevant advantages could include flexible developmental responses rather than a fixed adult trait. They would also depend on the maternal contribution and the conditions in which offspring develop. This would extend the earlier question about sex-specific effects of paternal capacities to ask whether their transmission can itself become sex-specific.

Evidence for persistent sexually antagonistic variation and models of recurring conflict and resolution give us reason not to assume that sex-specific regulation always provides a complete, permanent solution (Ruzicka et al., 2019; Pennell et al., 2016). The sharp divergence of male and

female lives at maturation, including potentially different demands on immune regulation of partnerships with rapidly-evolving microbial partners, may make Sierra domes especially revealing of conflicts that sex-specific gene expression does not fully resolve. I hypothesize that the difficulty of meeting those changing demands with shared genes that serve multiple functions could favor complementary ways of reducing the fitness costs of conflict, including regulated microbial assistance during development and discrimination over which paternal variants reach sons and daughters.

Such discrimination would require more than changing the brood's sex ratio. It would require a relationship between offspring sex and the other paternal variants transmitted, beyond the associations already imposed by the sex chromosomes, linkage, and ordinary inheritance. I would not assume that eggs already possess a male or female identity before fertilization. A change observed only among surviving young could instead arise through sex-specific developmental mortality. The comparison therefore needs to begin with the genetic and other semen-based contributions available from each male, and possibly contributions characteristic of each discernible male genre, following their representation through fertilization and subsequent offspring development. One possible benefit would be to reduce intralocus sexual conflict by favoring different paternal alleles or allele combinations in sons and daughters. Another would be to improve the fit between their inherited developmental and regulatory capacities and the microbial functions each sex can recruit and use. Neither hypothesized benefit would be established merely by finding a transmission bias. We need to know whether these biases are meaningful for whole brood fitness and subject to female choice.

This possibility could also help explain why females consistently choose to mate with only a **few** males out of the **much larger number** of willing suitors they encounter during their adult instar. Kekäläinen (2022) argues that within-male cryptic female choice could reduce the additional benefits of polyandry because meaningful genetic discrimination remains possible within a single male's ejaculate. In Sierra domes, a few well-chosen males might supply complementary contributions that can be used with considerable specificity. I hypothesize that filtering in the male, in the female, or at both stages could make the contribution ultimately realized from a given sire more functionally specific and predictable than a broadly sampled assortment of his possible meiotic products. Individual males, and potentially discernible male genres, might therefore offer characteristically different distributions of paternal alleles and allele combinations, including inherited capacities for recruiting and regulating useful microbial functions. Potentially transferred microbes and biochemical substances could add further dimensions to those offerings. Their value would depend on what they contribute to the particular female's brood: how they complement her own constitution and previously acquired contributions, and how they support the differing developmental and reproductive possibilities of her offspring, including sons and daughters.

The corresponding advantage in female assessment would arise if behavioral and chemical credentials make these differences sufficiently legible. A female could then obtain more of what she needs through a small number of discriminating choices, rather than depend as heavily on additional matings to acquire useful contributions whose availability is difficult to predict. The proposed benefit therefore involves both meaningful differentiation in what males offer and reliable information about those differences. Filtering within her reproductive system could further refine the contributions obtained from the males she accepts. This would make mate assessment and subsequent sperm discrimination complementary parts of a more effective and efficient process of assembling the brood—not simply two separate opportunities to favor generally superior males. An

additional male would then be valuable according to what he adds to the available possibilities, rather than simply because he adds another paternal identity. Filtering cannot create genetic variants absent from the accepted males, so between-male diversity and within-male discrimination could complement one another. Nor must stronger filtering always favor fewer mates: if it removes contributions useful to a particular female, another sire could become more valuable. The proposed explanation concerns an early decline in the marginal value of another mating when a small set of distinguishable males already supplies useful, complementary contributions.

The locally dense populations characteristic of Sierra domes may be particularly conducive to the evolution of this system. My earlier field study documented a midseason average of one inhabited web per 4.2 square meters, with some areas considerably more crowded (Watson, 1990). Where both sexes repeatedly encounter alternative prospective mates, a female can reject a male whose contribution adds little to what she already possesses, while a male rejected by one female may subsequently encounter another for whom his offering is valuable. Functional specialization could therefore pay without requiring every male to appeal equally to every female. Excessive specificity would nevertheless carry a cost if it made suitable recipients too scarce. I hypothesize that this balance could favor distinguishable but overlapping reproductive genres whose members offer related, reproductively useful contributions while retaining substantial individuality.

If such encounter opportunities recur across populations and generations, they could help sustain the evolutionary relationship between differentiated male contributions and female mechanisms for recognizing and using them. Females could benefit from obtaining a complementary collection efficiently, while males could benefit from developing and advertising contributions that enough females repeatedly find useful. The proposed system might consequently become especially well developed in species whose ecology reliably supplies these opportunities. This predicts a stronger correspondence among male developmental organization, behavioral and chemical credentials, and the contributions ultimately realized in particular females' broods—not simply more matings at higher density. Comparisons among populations differing in encounter opportunities would provide a way to investigate that prediction. This gives us a reason why Sierra domes might be especially revealing beyond their exceptional observability: their ecology may favor particularly strong development of the proposed system. Their density may help sustain the very evolutionary relationships this program is trying to explain.

The female's immediate circumstances remain part of this explanation. My observations distinguish the foraging advantages she obtains during prolonged copulatory courtship from the conditions prevailing during precopulatory courtship or when a male is present but attending primarily to his own feeding. A female can interrupt copulatory courtship by moving toward the periphery of the dome, without necessarily leaving it altogether; the male's readiness to continue also varies. A hungry male concentrating on feeding rather than courtship may be especially effective at appropriating prey from her web. These differences mean that the value of another encounter cannot be represented by a single mating cost. The genetic opportunity would have to be considered alongside the encounter's nutritional consequences, time demands, predation exposure, and potential transmission of pathogens. The proposed filtering mechanism would supply an additional reason why the genetic return from further mating might diminish after a small number of useful contributions have been obtained.

The existing study provides several starting points without requiring this entire account to be tested at once. Repeated behavioral histories can be paired with accessible secretions, excreta, and fresh ejaculates from known males. With suitable molecular methods, future work could compare

paternal alleles and combinations in available sperm, functionally differentiated sperm fractions, and the earliest accessible offspring stages, followed by later survival and reproductive performance. Comparisons would also need to retain the maternal genetic contribution: compatible matching between particular paternal and maternal combinations could occur even without an overall change in paternal allele frequencies across the brood. Experiments changing a microbial association or its products could then ask whether they change sperm performance alone or also change the representation of paternal genetic variants. The larger question is whether the processes that make a male a particular reproductive individual also help determine what he can contribute to a particular female's brood. These are connected opportunities for future investigators, not additional prerequisites for continuing the present field study.

Testing structured individuality on the female side

A linked study would follow identified females as well as characterized males. Repeated encounters across a range of male combinations would separate persistent differences among females from changes within the same female as her condition and mating history develop. The sequence and timing of those encounters would remain part of the record, because an earlier male could change a female's microbial or physiological circumstances even if he ultimately contributes little genetic paternity. The informative question is not only which males do well on average, but whether their relative value changes predictably with the recipient.

Females that look similar in mate acceptance could differ in sperm use or offspring outcomes. Chemical manipulations paired with characterized performances could then ask whether the same cue has different effects in those females. Repetition across seasons would reveal whether individual patterns recur around broader population-level themes and whether those themes correspond to recurring male combinations.

The analysis should not require a few rigid female types. It should allow overlapping preference patterns and changes in their expression. Finding repeatable differences would establish one level of organization; relating them to reproductive consequences would establish another. Inheritance studies, ecological comparisons, and explicit evolutionary models could then investigate how that organization persists or changes across generations. None requires one student to establish the entire coevolutionary account.

Testing immune-state-dependent sire weighting

The immune-state-dependent sire-weighting hypothesis requires a further connection: an independently characterized change in the female's own reproductive health. At present, I cannot reliably tell from a non-virgin female's responses to suitors whether disease is changing her choosiness. Those responses are naturally variable. Some gravid females become sluggish shortly before death, but that late sign does not identify the earlier physiological state in which the proposed compensatory response might operate. Establishing informative health measurements is therefore part of this investigation, not an ability the study already possesses.

A practical starting point is to follow identified females through their encounters and then document their reproductive outcomes without diagnosing health from mate acceptance itself. Known females must be collected before they leave their webs to oviposit; otherwise, the history usually ends in disappearance into the forest. In forty-five years, I have followed that departure through successful oviposition in nature only once. The marked female descended to the forest floor and traveled about fifty feet before selecting her site, building the bottom of her cocoon, laying

normal eggs, and closing the cocoon over them. Most of what we know about the laying outcomes of individual study females comes from bringing them into captive care before that departure.

Under those observable conditions, a female may start the bottom half of her cocoon and then fail to lay, becoming lethargic and dying soon afterward. Another may complete laying but produce a mixture of normal and hardened, inviable eggs. These outcomes can be connected retrospectively to preceding courtship and mating histories, while remaining distinct from successful production of viable young. The outcome alone would not establish when a difficulty began or what caused it. Independent physiological, pathological, and microbial evidence would be needed to investigate that connection and develop ways of recognizing relevant states earlier in life.

As those measurements become available, repeated encounters could ask whether a particular combination of male performance and chemical information gains or loses relative influence as the female's condition changes. Female age, feeding history, prior matings, infection evidence, and egg development would remain attached to each record. Comparisons could distinguish responses associated with infection from those associated with nutritional difficulty, generalized sickness, or changing receptivity. The theoretical interest lies in the altered value of a particular paternal contribution, not simply in a reduction in mating caused by illness.

Paired chemical and behavioral tests could then ask whether chemistry adds information beyond the performance alone. Initial acceptance, completion of the separate mating phases, sperm representation, fertilization shares, and subsequent offspring viability would remain distinguishable outcomes. Including females that retain effective reproductive functioning as well as those approaching severe decline would help separate compensatory evaluation from impaired assessment. The proposed benefit concerns offspring the female remains capable of producing: an inherited paternal contribution is not a treatment for her already failing maternal tissues or eggs. Its value must ultimately be tested in the offspring, where the next part of the program follows it.

Following contributions into offspring and across generations

The strongest evolutionary test asks what the changed paternal contributions accomplish for offspring under relevant circumstances. The 1998 growth study provides a foundation, but early growth is not the only informative outcome. Juvenile survival, sons' adult performance trajectories, and daughters' ability to sustain viable eggs through oviposition would reveal whether a paternal contribution has different consequences at different stages and in the two sexes.

Those outcomes belong within the whole-brood question. We need to know not simply whether one class of offspring improves, but how different contributions affect the brood's combined reproductive prospects. Where diversification is proposed to reduce shared vulnerability, the relationships among offspring responses to the same challenge become important alongside their average performance. Controlled rearing comparisons and relevant microbial environments could test when different contributions are useful and when a proposed mixture offers no benefit.

Offspring comparisons would retain the connection to each sire as a known, developmentally characterized individual. His characteristic behavior and possible reproductive genre would be considered together with his physical and physiological capacities, host regulation, microbial associations and functions, and the chemistry through which some of those relationships might become perceptible to a female. *I want to understand whether that integrated organization predicts what he actually contributes, and what those contributions allow particular offspring to accomplish under their own circumstances.*

Where the genetic resolution permits, the investigation would follow not only the identity and share of each sire but also the particular paternal alleles and combinations of alleles inherited within his contribution. This would let us ask whether the same processes that help construct a male's reproductive phenotype also influence which of his possible haploid genetic contributions become available in an ejaculate, and whether the receiving female further changes their representation. One especially consequential possibility is that different contributions from the same sire could be disproportionately represented in sons and daughters in ways that support their different reproductive requirements, while also contributing to flexible developmental responses within each sex.

Parent–offspring work would also distinguish inherited host capacities, parental or microbial transmission effects, and compatibility with the female's constitution. Offspring resemblance alone would not separate them. A useful inherited contribution could be regulatory responsiveness that helps offspring construct an effective phenotype under their own conditions, rather than a copy of the father's mature behavior or microbiome.

Parental microbial effects can also operate through changes in parental physiology or substances supplied to developing offspring, without requiring the microorganisms responsible to establish in the offspring (Comizzoli et al., 2021). In Sierra domes, identifying such developmental influences would help us ask how inherited regulatory capacities are expressed under different microbial and nutritional starting conditions.

Microorganisms and biochemical substances transferred during reproductive encounters would be investigated as potentially distinct contributions, including possible effects on the female's reproductive environment and the conditions under which offspring begin development. These effects need not coincide with the donor's genetic share of paternity. Following paternal genetic representation from the earliest accessible reproductive stages through later development would help distinguish filtering before or at fertilization from changes caused by subsequent, potentially sex-specific survival.

The archived continuation proposal envisaged mother–daughter comparisons of mate and sire preference functions. Renewing that direction would connect the consequences of female evaluation with its cross-generational continuity. A staged design could ask whether recurring preference patterns are inherited, reconstructed through development, or both, and whether their consequences help explain why those patterns persist. The original projected samples were plans, not completed datasets, but the female-centered question remains a substantial opportunity (Watson, archived NSF continuation proposal, PDF pp. 7–8, 13, 16).

A separate opportunity: skipping by associative females

Alongside the central personality and mating-system metagenomics programs, a narrower ongoing study concerns skipping by associative females—a display whose function I do not yet confidently understand. The skipping display is very distinctive, involving one or more quick one- to two-second bouts of leg movements that remind me of energetic Irish step dancing. All eight legs take part simultaneously. A female can perform it in place or while moving across the dome. Its occurrence is far from indiscriminate. My repeated observations show females orienting toward two males on the web and sometimes approaching while skipping. An intensely displaying female may get right in their faces. My strong impression is that she does this especially when the males are futzing around rather than engaging in a demanding contest. She commonly stops once vigorous fighting begins. An associative female alone with her guard almost never skips, even when he actively probes her. This

suggests an intervention that initiates or intensifies competition, distinct from the sexual-receptivity signal given by skipping mature, non-virgin females. An associative female has not yet matured and cannot proceed to mating.

An already extensive unpublished manuscript can be strengthened with substantial additional field observations collected during 2018–2023. We staged fights between males matched as closely as practicable in body mass, using two observers, a stopwatch, and detailed data sheets to record the sequence and timing of male and female behaviors. These records can test when skipping occurs, what the males do immediately afterward, and whether skipping frequency and intensity increase as the female approaches her retrospectively known maturation time. My observations of stalled encounters becoming active after skipping motivate those analyses; they are not a completed causal demonstration.

A more specific hypothesis is that skipping reduces an informational asymmetry between a longtime guard and a newcomer concerning the female's proximity to maturation, without revealing a precise countdown that might discourage sustained guarding. The existing field records cannot adequately resolve that explanation. One or two seasons of focused work with captive spiders could control the males' histories with a female and use our established observation methods to examine the resulting displays and contests. An additional capable assistant or collaborator could carry that investigation forward with my guidance and earn authorship through a substantive contribution. The completed fieldwork offers a publication opportunity in its own right; this experimental continuation is separate from, and not a prerequisite for, the larger programs. This would also provide an opportunity to teach the methods and investigate the unresolved display in the living system.

Another project-in-waiting: facial and cheliceral fluctuating asymmetry

Another unusually rich research opportunity survives in hundreds of close-up black-and-white photographs taken during the foundational field studies. My morning laboratory measurements of collected males ended with carefully prepared frontal and rear photographs of their faces and mouthparts against a stage micrometer. These reveal the arrangement of the eyes, the paired chelicerae, and the intricate tooth-bearing margins and fangs. Every male represented in this collection had been observed in the field either mating with a virgin female as her champion first mate or courting a non-virgin female, successfully or unsuccessfully. Body measurements and the observed reproductive encounter belong to the same individual as the photographs. For every male that mated, we also recorded standardized copulatory-courtship hit rates; for a small, particularly valuable subset, we have estimates of fertilization success. These are therefore much more than anatomical portraits: each face belongs to a male whose actual reproductive performance we watched. The original negatives remain stored in their Kodak tubes, awaiting systematic high-resolution digitization to assemble the full collection for analysis.

The proposed investigation would ask whether facial and cheliceral fluctuating asymmetry—the small, randomly directed departures from bilateral symmetry—reveals an aspect of developmental stability associated with reproductive performance. A recent study of *Tetragnatha extensa* demonstrates that photographic geometric morphometrics can detect differences in spider asymmetry associated with developmental conditions even when corresponding differences in body size are absent (Mutel et al., 2026). The Sierra dome archive offers the further opportunity to connect such measurements directly to naturally occurring sexual selection. Do champion first mates differ from secondary suitors in the symmetry or shape of particular structures? Among males courting non-virgin females, do accepted and rejected suitors differ after accounting for body

size and the circumstances of their encounters? Among males that mate, are these morphological characteristics associated with copulatory-courtship performance and, in the select subset with paternity estimates, fertilization success? The hypothesis does not require females to inspect a male's face visually. An association could instead motivate investigation of developmental capacities expressed jointly in anatomy, mechanical performance, and chemical characteristics available to female assessment. The photographs would provide a new way to investigate the same individuals whose behavior and reproductive outcomes established the original mating-system findings.

Modern methods make this a more approachable project than it was when the photographs were taken. The program ML-morph provides a demonstrated machine-learning approach to locating biological structures and placing anatomical landmarks, with accuracy comparable to manual annotation in the standardized datasets tested (Porto & Voje, 2020). Work on human facial photographs also provides a methodological precedent for separating orientation-related distortion from anatomical asymmetry, rather than treating every apparent left–right difference as biological (Weiss et al., 2020). These approaches would be adapted and validated for the spider preparations, whose positioning was carefully standardized but retains small differences in rotation and tilt. The first stage would identify reliably measurable landmarks, quantify measurement and orientation uncertainty, and establish criteria for retaining images or particular structures before examining their reproductive associations. Where defensible, individual asymmetry estimates could carry uncertainty bounds, with the best-positioned faces providing a particularly useful initial sample. The aim would be to recover anatomical information, not digitally beautify the males into symmetry. With appropriate morphometric collaboration, a capable undergraduate or other investigator could develop a focused publication from this remarkable collection: new measurements of the very males whose courtships, matings, and sometimes paternal contributions were documented decades ago.

A career can begin with one connected question

Students, collaborators, and successors would learn to recognize biological states, handle the animals without disrupting the behavior of interest, and connect laboratory measurements to natural encounters. They could begin with a well-defined behavioral, physiological, anatomical, or molecular question and make a publishable contribution without first acquiring every technique described here. The larger program gives that contribution a context and a route toward further questions.

A successor interested in chemical communication could begin with the information added by a characterized odor. Another could concentrate on the development of individuality, the unfinished behavioral-energetics analyses, reproductive anatomy, or host regulation of microbial functions. A collaborator interested in reproduction and conservation could investigate how parental microbial functioning and the offspring's early environment influence the expression of inherited capacities. These are routes into the central program, not obligations to complete a catalogue of my unfinished ideas. There is far more in the system than this prospectus attempts to enumerate.

The broader contribution is an approach to sexual-selection research organized around the development of an individual contribution and its evaluation by an individual recipient. The exceptional observability of Sierra domes allows those two sides to remain connected to natural biology. The questions can inform other mating systems without assuming that the same reproductive genres, cues, or mechanisms occur everywhere.

Continuity would not require intellectual conformity. I want successors to have the knowledge and experimental access with which to discover where the present account is right, where it is

incomplete, and where the spiders demand a different explanation. Over the next two or three decades, independent investigators could carry this inquiry much further than I can take it alone. The scientific inheritance would be a living system and the ability to ask better questions of it.

The rate at which these opportunities proceed alongside one another will depend chiefly on the availability and quality of collaborators and assistants. I plan to meet the ordinary field-program costs and much of the equipment expense myself. A salaried postdoctoral collaboration would require separately obtained grant support, and an investigator willing to help develop that proposal would be especially valuable. The prospectus describes work that can grow over decades, not a timetable requiring one person to execute all of its methods in the next few summers.

The field and laboratory investigations would remain linked through the identities and histories of the spiders studied. Sequencing, biochemical assays, epigenomics, imaging, and specialized chemical work can each address a focused question about those individuals. Their coordination provides a way to connect development and performance with female responses and offspring outcomes as collaborators, methods, and resources become available.

6. Comparative research and scientific collaboration

Comparisons among populations

The FLBS research documents a population within a known habitat, prey community, and seasonal history. Local host–microbe relationships are now part of the question. Comparisons with other populations could draw on that record to ask which aspects of developmental organization, female evaluation, and reproductive outcomes recur under different ecological and microbial circumstances.

The retained records provide questions for both further analysis and new experiments. They can guide tests of earlier findings, the collection of new developmental histories, and refinement of the observational methods. Field training gives new investigators practice in recognizing the animals' states and interpreting their encounters in the circumstances in which they occur.

Female physiological self-assessment, locally useful microbial associations, and the value of different sire contributions can be examined as relationships that may vary among populations and seasons. The program's male-personality observations, physiological records of individual differences and decline, and approaches to chemical information and female responses offer complementary starting points. The purpose of comparative work is to identify both recurring relationships and differences requiring their own explanation.

The new molecular opportunities make detailed individual histories especially informative. A sophisticated assay on an anonymous specimen answers a different question from the same assay linked to a known male's development, courtship, competitors, and female responses. The value lies in connecting a molecular observation with what the particular animal has done and what it subsequently accomplishes, rather than treating every specimen as biologically interchangeable.

I hope to develop collaborations and proposals that combine practical field training with specialized methods. My UNM colleague Kelly B. Miller visited the population in 2024, and we have discussed collaboration and graduate-student involvement. I continue to seek additional collaborators and even successors.

Learning the system and developing independent studies

Investigators can become involved through field observation and experiment, analysis of retained records, or a focused physiological, chemical, molecular, or anatomical question. My existing instruments and equipment, the retained materials, and practical methods described here provide several ways to begin. The combination chosen would follow the scientific question and the investigator's interests and expertise.

Specific projects, supervision, funding, and field or laboratory arrangements would be agreed individually before participation. This prospectus describes research opportunities rather than confirmed placements.

Continued investigation can reveal further significance in earlier findings, connect them to mechanisms not previously understood, and increase their usefulness for subsequent research. It can also address questions that the prior work opened but could not itself resolve. Realizing this potential requires new observations and experiments, together with the training of successors who can learn the methods and natural history of this living system and develop investigations of their own.

I invite scientific colleagues to consider the questions in this prospectus in relation to their own interests and methods. Some may find a focused collaboration; others may see possibilities for an independent investigation. The question is what new knowledge this system can still produce.

For a prospective successor, the immediate invitation is a scientific conversation. This prospectus cannot contain everything learned in forty-five years, and it should not prescribe every future question. I want it to show enough of the system, the evidence, and the possibilities to make an investigator want to see the animals, discuss a project, and consider what they could discover here. My hope is that such conversations lead to field experience, collaboration, and eventually independent research lives.

Appendix A. Competitive support and durable investment

The program combines competitive public support, long-lived equipment, station-enabled technical training, and sustained personal investment. Award identifiers and terms are summarized from the program record.

Support	Contribution to the program
Graduate research at Cornell	NSF Doctoral Dissertation Improvement Grant BSR-8311331; NIH National Research Service Award support under 5T32MH15793; three Sigma Xi grants-in-aid from the national and Cornell chapters (Watson, 1993); Cornell Laboratory of Ecological and Evolutionary Genetics; Cornell Section of Neurobiology and Behavior.
NSF Environmental Biology postdoctoral support	Competitively selected for a two-year fellowship. A technical degree-conferral eligibility ruling limited actual support to one year, administered as a supplement to Randy Thornhill's grant BSR-8515377. This supported my independent Sierra dome research, not that grant's subject.
NSF–NATO postdoctoral fellowship	RCD-8854490, University of Oxford, under William D. Hamilton. The initial twelve months were extended by four months, for sixteen months of support. Oxford Zoology provided additional institutional support.
Sole-PI NSF research grant	IBN-9321326, "Courtship Energetics and the Heritability of Metabolic Competence"; $60,000; 1994–1996. Supported continued research and the Sartorius microbalance and Sable Systems respirometry infrastructure.
Continuing personal and family investment	Substantial expenditure over subsequent decades on assistants, travel, housing, equipment, and supplies. This sustained the program after the federal awards ended.

Equipment and training that continue to support research

The microbalance remains part of routine opponent selection and physiological measurement. The respirometry system supported the published fighting study, the larger copulatory-courtship investigation, bumblebee respiration-mode studies, and collaborative research on the energetic costs of sexual conflict in water striders (Watson, Arnqvist, & Stallmann, 1998).

More recent molecular work benefited from FLBS laboratory access and extensive hands-on training by Logan Peoples, with station support associated with Jim Elser and Matt Church. That technical foundation is part of the program's present capacity.

That recent training built on earlier microbiological and cell-culture research experience at UNM, including work with Oswald Baca, and at Oxford's NERC virology laboratory during my postdoctoral period with W. D. Hamilton. Identifying agents of the spider's reproductive disease has been a longstanding experimental objective. The more recent candidate-directed assays and commercial pooled bacterial survey represent completed exploratory steps toward that objective, with laboratory details and results retained in the research record.

Appendix B. Published foundation and selected retained studies

Older publications use *Linyphia litigiosa*; later papers use *Neriene litigiosa*. The nine papers below concern the same Sierra dome study system.

Watson, P. J. (1986). Transmission of a female sex pheromone thwarted by males in the spider *Linyphia litigiosa*. *Science, 233*, 219–221.

Watson, P. J. (1990). Female-enhanced male competition determines the first mate and principal sire in the spider *Linyphia litigiosa* (Linyphiidae). *Behavioral Ecology and Sociobiology, 26*, 77–90.

Watson, P. J. (1991a). Multiple paternity and first mate sperm precedence in the sierra dome spider, *Linyphia litigiosa* Keyserling (Linyphiidae). *Animal Behaviour, 41*, 135–148.

Watson, P. J. (1991b). Multiple paternity as genetic bet-hedging in female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae). *Animal Behaviour, 41*, 343–360.

Watson, P. J. (1993). Foraging advantage of polyandry for female sierra dome spiders (*Linyphia litigiosa*: Linyphiidae) and assessment of alternative direct benefit hypotheses. *The American Naturalist, 141*, 440–465. doi:10.1086/285483

Watson, P. J., & Lighton, J. R. B. (1994). Sexual selection and the energetics of copulatory courtship in the sierra dome spider, *Linyphia litigiosa*. *Animal Behaviour, 48*, 615–626.

Watson, P. J. (1998). Multi-male mating and female choice increase offspring growth in the spider *Neriene litigiosa* (Linyphiidae). *Animal Behaviour, 55*, 387–403.

deCarvalho, T. N., Watson, P. J., & Field, S. A. (2004). Costs increase as ritualized fighting progresses within and between phases in the sierra dome spider, *Neriene litigiosa*. *Animal Behaviour, 68*, 473–482.

Keil, P. L., & Watson, P. J. (2010). Assessment of self, opponent and resource during male–male contests in the sierra dome spider, *Neriene litigiosa*: Linyphiidae. *Animal Behaviour, 80*, 809–820.

Related publication

Watson, P. J., Arnqvist, G., & Stallmann, R. R. (1998). Sexual conflict and the energetic costs of mating and mate choice in water striders. *The American Naturalist, 151*, 46–58. doi:10.1086/286101

Selected unpublished material described in this prospectus

I retain the raw data from every study I have conducted. These records preserve the basis for verification of the published findings and permit further analysis where questions remain. The historical paternity and behavioral-energetics collections remain distinct from the recent personality cohorts; earlier measurements gain further value when their questions can be revisited through new experiments in the population from which they arose. The entries below identify selected retained materials supporting this prospectus, not the full range of possible future projects.

Longitudinal and archival collections. Multiple years of copulatory-courtship behavioral-energetics data, including repeated observations on individual males throughout their lifespan with background behavioral data from the field, e.g., maturation date, body mass, first mate (“champ”) versus secondary mating status, longevity in captivity; the main 2024–2025 personality studies; and associated specimens frozen in molecular-grade EtOH, behavioral records, and observational methods. Season-specific coverage and specimen links will be indexed without conflating separate datasets. The confirmed ethanol-preserved material is from recent work; availability of older bodies originally frozen during the energetics studies remains to be checked. This uncertainty does not concern the retained raw behavioral and physiological data.

Additional retained studies. The associative-female skipping manuscript and detailed 2018–2023 staged-fight observations include timed records of both male and female behavior. The 2025 Polytec vibrometry proof of concept produced saved files identified by male, with some time-linked handwritten annotations; it was a feasibility pilot, not a repeated-measures study of individual signaling styles.

Facial and cheliceral photographic archive. Hundreds of paired frontal and rear black-and-white photographs, retained as original negatives, are linked to males observed as champion first mates or secondary suitors. The associated records include copulatory-courtship hit rates for mates and fertilization estimates for a select subset. Section 5 describes digitization and the proposed morphometric investigation.

Historical sources for the physiological and female-preference framework

Watson, P. J. (undated). Archived NSF continuation proposal, MET7b3.pdf (30-page working copy). The repeated-measures study is described on PDF p. 2; the individual female preference-function design on pp. 7–8; preliminary metabolic-decline results in Figs. 3–4 and accompanying text on pp. 10–11; respirometry processing on p. 12; and operational definitions and repeatabilities in Table 1 on p. 15. Detailed fighting-ferocity results occur in working notes on p. 28. Here cited as "archived NSF continuation proposal." The file contains draft placeholders and prospective work; its exact submission date is not established, and proposed cross-generational studies are not treated as completed findings.

Watson, P. J. (undated). Final Report – NSF Award 9321326. Retained final report. The report documents copulatory-courtship energetics in approximately 165 males over 1994–1998, with two to five repeated measurements for many males, and identifies the collection as incompletely analyzed and unpublished. This larger collection is distinct from the 24-male 1994 subset analyzed in the continuation proposal and from the 2024–2025 personality cohorts.

Additional firsthand research account, 4 October 2026. The author's discussion records the first-mate substitution interpretation; the distinction between fighting ferocity and fight-winning ability; preliminary observations of somewhat shorter lifespans among high early metabolic performers; the detailed courses of the two female egg-failure syndromes; and the new immune-state-dependent compensatory sire-choice hypothesis. The longevity observations and pathological descriptions are not presented as new statistical analyses of the proposal's data. Published findings, archived preliminary analyses, current firsthand observations, proposed measurements, and new explanations remain separate evidential categories.

Watson, P. J. (2026). Additional firsthand research accounts recorded in the October 5–8 prospectus discussions. These describe reproducible collection of fresh semen from selected mature males, two ejaculate-deposition opportunities, the absence so far of demonstrated semen-associated microbes, and an exploratory pooled bacterial survey using Zymo host-DNA depletion and commercial analysis. The accounts distinguish repeatedly observed natural history from approximate sample totals and assay particulars retained in laboratory notebooks.

Watson, P. J. (2026). Additional firsthand research account, 16 October 2026. Retained prospectus discussion documenting the hit-and-flub observation method, archival and specimen availability, the 2025 vibrometry pilot, secretions and excreta collection, reproductive-failure observations, and the ongoing associative-female skipping study. It distinguishes completed observations and collection methods from proposed analyses and experiments.

Completed exploratory molecular work. Individual-spider DNA extractions and targeted bacterial screening, including work on spiders with known behavioral histories, were complemented by a pooled-body metagenomic investigation in 2021. Retained laboratory notes document HostZERO preparation, DNA quantification, and concentration before commercial sequencing. Logan Peoples advised on preparation and performed the subsequent computational analyses, identifying bacterial ribosomal sequences and reconstructing substantial portions of several bacterial genomes. The retained results and his explanatory email of 28 August 2021 document the principal bacterial assignments, genome-quality assessments, and opportunities for further taxonomic and functional investigation. These materials provide a foundation for continued individual-spider studies connecting microbial associations to behavior, physiology, and reproduction.

Urlacher–Watson study (2007). *Increased copulatory courtship effort in matings with females of lower reproductive value by male sierra dome spiders (Neriene litigiosa).* Unpublished manuscript and retained raw observations; analysis and reporting require reassessment.

Mating context and energetic investment. A 2007 study with Samuel S. Urlacher, supported through the FLBS NSF REU program, compared aspermic courtship with virgin and nonvirgin females while recording metabolism and behavior. Its unpublished manuscript reports preliminary differences in cumulative investment and associations with female mass, which is correlated with expected clutch size and thus female reproductive value. The successful repeat-mating sample involving non-virgin females was small and the analyses need checking against the retained data. The opportunity is specific: test how investment changes when the route to sire success differs between first and secondary matings—not treat the preliminary estimates as settled.

Lactate pilot (1996). Respirometry-linked endpoint freezing and spectrophotometric assays; original results retained by the author. The linked measurement design is documented in the original 1996 recording form.

Biochemical costs of terminal performance. In a 1996 pilot, I monitored copulatory courtship in the respirometry system, rapidly froze males in liquid nitrogen near its end, and used spectrophotometric lactate assays to investigate a possible “maximal final effort” anaerobic contribution to courtship performance. These were completed observations and assays, with results retained. I recall a small association near the assay’s sensitivity limits; modern reassessment and replication are needed. The pilot already connects a known behavioral endpoint to physiological output and preserved tissue.

Appendix C. Selected comparative and conceptual references

These sources support the comparative context and candidate mechanisms, not their occurrence in the FLBS population. Rowe et al. (2020) provide an evolutionary framework linking reproductive microbiomes with sexual selection and mating systems. Comizzoli et al. (2021) place microbial functions within the reproductive life cycle and conservation of wild animals. A recent meta-analysis documents experimental microbiome effects on behavior with substantial variation among studies (Schetini de Azevedo et al., 2026). The program's own publications are listed in Appendix B.

The within-sire extension draws on evidence for haploid-biased gene expression (Bhutani et al., 2021), genetic and offspring-performance differences associated with sperm subpopulations (Alavioon et al., 2017), and haplotype-specific gamete matching (Lenz et al., 2018), together with the theoretical treatment of within-male cryptic female choice by Kekäläinen (2022). These sources motivate the proposed connection to developmental organization; they do not demonstrate the microbial filtering mechanism proposed here.

Alavioon, G., Hotzy, C., Nakhro, K., Rudolf, S., Scofield, D. G., Zajitschek, S., Maklakov, A. A., & Immler, S. (2017). Haploid selection within a single ejaculate increases offspring fitness. *Proceedings of the National Academy of Sciences, 114*(30), 8053–8058. doi:10.1073/pnas.1705601114

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Briggs, J. R., Espindola, C., & Krugner, R. (2026). Mating communication signals of the blue-green sharpshooter, *Graphocephala atropunctata*. *Journal of Insect Behavior, 39*, 32. doi:10.1007/s10905-026-09926-0

Comizzoli, P., Power, M. L., Bornbusch, S. L., & Muletz-Wolz, C. R. (2021). Interactions between reproductive biology and microbiomes in wild animal species. *Animal Microbiome, 3*, 87. doi:10.1186/s42523-021-00156-7

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Kekäläinen, J. (2022). Cryptic female choice within individual males – A neglected component of the postmating sexual selection? *Journal of Evolutionary Biology, 35*(11), 1407–1413. doi:10.1111/jeb.14081

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