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Les mécanismes génétiques et évolutifs impliqués dans la variation phénotypique d'un trait exagéré sous sélection sexuelle

Genetic & evolutionary mechanisms underlying phenotypic variation in an exaggerated sexually selected trait

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« *Cela fait partie du rituel* » Birzim Scantius - LBA

« *J'optimisme* » Mme. Cresson

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Abstract

Phenotypic variation results from the interplay between genetic, environmental factors and their interaction, constituting the foundation on which selection acts. Understanding how phenotypic variation is generated, altered and maintained is therefore a central question in evolutionary biology. Exaggerated sexually selected traits, such as weapons and ornaments, are among the most variable phenotypes observed in nature. The evolutionary mechanisms responsible for the maintenance of such extreme variation despite strong directional selection remain poorly understood. Several models have been proposed to explain the maintenance of high variation in sexually selected traits, however, there are only a few empirical studies testing these models. The water strider *Microvelia longipes* provides a powerful model to investigate this paradox. Males have evolved an exaggerated third pair of legs used as weapons during male–male competition, and leg length varies extensively within natural populations. This thesis used life-history experiments and a combination of experimental evolution and population genomics to investigate the mechanisms underlying this extreme variation. First, I tested whether the maintenance of exaggerated legs induces viability costs in adult males. Survival analyses revealed no detectable survival cost associated with leg length. Instead, male survival was primarily determined by body size, suggesting that exaggerated weapons do not reduce lifespan once developed. Then, to identify alleles associated with variation in leg size and to characterise the phenotypic and genomic responses to sexual selection, we used an Evolve & Resequence (E&R) approach. Experimental populations were subjected to directional selection for dominant males over 15 generations. At multiple time points, individuals from both selection and control lines were phenotyped for body size and leg length, and pools of 50 individuals per population were sequenced. This design allows us to monitor both phenotypic evolution and allele frequency changes across generations. Phenotypic data show a clear and rapid response to selection: males from the selection lines exhibit increased leg size compared to the controls. Interestingly, phenotypic variation remained high in the selection lines despite persistent directional selection, whereas it decreased in the controls. These patterns suggest that sexual selection seems to maintain rather than eroding it. We further demonstrate that selection favouring long-legged males generates substantial fecundity costs in females. These fitness costs suggest that genetic variation may be maintained through unresolved conflict between the sexes, preventing fixation of alleles associated with extreme trait exaggeration. Together, these results show that directional sexual selection can promote trait exaggeration while maintaining substantial phenotypic variation. Ongoing genomic analyses will determine the genetic diversity associated with selection.

Résumé

La variation phénotypique résulte de l'interaction entre les facteurs génétiques, les facteurs environnementaux et leurs interactions, constituant le substrat sur lequel agit la sélection. Comprendre comment cette variation est générée, modifiée et maintenue représente ainsi une question centrale en biologie évolutive. Les caractères soumis à la sélection sexuelle, tels que les armes et les ornements, figurent parmi les phénotypes les plus variables observés dans la nature. Pourtant, les mécanismes évolutifs permettant le maintien d'une telle variabilité malgré une forte sélection directionnelle demeurent encore mal compris. Plusieurs modèles théoriques ont été proposés pour expliquer le maintien d'une forte variation des caractères soumis à la sélection sexuelle, mais peu d'études empiriques ont permis de les tester. La punaise d'eau *Microvelia longipes* constitue un modèle particulièrement pertinent pour étudier ce paradoxe. Les mâles possèdent une troisième paire de pattes postérieures exagérément développées, utilisée comme arme lors des compétitions entre mâles, et la longueur de ces pattes présente une très forte variabilité au sein des populations naturelles. Cette thèse combine des expériences d'histoire de vie, une approche d'évolution expérimentale et des analyses de génomique des populations afin d'étudier les mécanismes responsables du maintien de cette variation extrême. Dans un premier temps, j'ai évalué si le développement de pattes exagérées engendrait un coût de viabilité chez les mâles adultes. Les analyses de survie n'ont révélé aucun coût détectable associé à la longueur des pattes. En revanche, la survie des mâles dépendait principalement de leur taille corporelle, suggérant que les armes exagérées ne réduisent pas la longévité une fois leur développement achevé. Dans un second temps, afin d'identifier les variants génétiques associés à la variation de la taille des pattes et de caractériser les réponses phénotypiques et génomiques à la sélection sexuelle, nous avons mis en œuvre une approche d'évolution expérimentale couplée au re-séquençage des populations (Evolve & Resequence). Des populations expérimentales ont été soumises à une sélection directionnelle favorisant les mâles dominants pendant 15 générations. À plusieurs générations, les individus des lignées sélectionnées et des lignées témoins ont été phénotypés pour la taille corporelle et la longueur des pattes, puis des pools de 50 individus par population ont été séquencés. Ce dispositif permet de suivre l'évolution des phénotypes et les changements de fréquences alléliques au cours de l'expérience. Les données phénotypiques mettent en évidence une réponse rapide et marquée à la sélection : les mâles des lignées sélectionnées développent des pattes significativement plus longues que ceux des lignées témoins. De manière remarquable, la variation phénotypique demeure élevée dans les lignées sélectionnées malgré une sélection directionnelle continue, alors qu'elle diminue dans les lignées témoins. Ces résultats suggèrent que la sélection sexuelle pourrait contribuer au maintien de la variation phénotypique plutôt qu'à son érosion. Nous montrons également que la sélection favorisant les mâles aux longues pattes engendre des coûts importants sur la fécondité des femelles. Ces coûts de valeur sélective suggèrent que la variation génétique pourrait être maintenue par un conflit sexuel non résolu, empêchant la fixation des allèles associés à l'exagération du caractère. Dans leur ensemble, ces résultats montrent que la sélection sexuelle directionnelle peut favoriser l'exagération d'un caractère tout en maintenant une importante variation phénotypique. Les analyses génomiques actuellement en cours permettront de déterminer dans quelle mesure cette réponse évolutive s'accompagne de changements de la diversité génétique et d'identifier les régions du génome ciblées par la sélection.

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Introduction

I. Adaptation and the importance of phenotypic variation

The extraordinary diversity of life on Earth has fascinated biologists for centuries. Organisms differ dramatically in their morphology, physiology, behaviour, or life-history strategies, occupying a vast range of ecological niches and environments. Understanding how such diversity originates and evolves has therefore long been a central objective of evolutionary biology. A major breakthrough in addressing this question came with Darwin's theory of evolution by natural selection (Darwin, 1959), which emphasised that the diversification of species depends on variation among individuals within populations. Rather than being composed of identical individuals, populations exhibit substantial variation in traits such as body size, colouration, behaviour, physiology, and reproductive strategies. These observable differences among individuals constitute phenotypic variation. Within species, populations experiencing contrasting environmental conditions may evolve distinct phenotypes, giving rise to locally adapted ecotypes (Gregor, 1944). One example is the field mugwort (*Artemisia campestris*, Asteraceae), which occurs across a wide range of habitats. In Sweden, populations growing on shifting sand dunes exhibit broad leaves, white hairs and an upright growth form, whereas populations present in calcareous rocky habitats display horizontally spreading branches and lack an upright growth form (Turesson, 1925). Phenotypic variation can also occur between the sexes. Sexual dimorphism generates differences between males and females that rival, and sometimes exceed, those observed between closely related species. For example, male peafowl (*Pavo cristatus*) possess an iridescent blue and green plumage as well as an exaggerated tail, known as the train, which is marked with numerous eyespots. In contrast, peahens have a dull grey-brown plumage and completely lack this exaggerated tail (Darwin, 1959).

Phenotypic variation becomes a target of natural selection when traits are heritable and differ in their effects on fitness, defined as an individual's ability to pass its alleles on to the next generation (Kimbrough, 1980). Under these conditions, natural selection favours phenotypes that increase fitness, thereby altering trait frequencies across generations (Darwin, 1959). More broadly, natural selection encompasses any consistent difference in survival or reproductive success associated with phenotypic differences among individuals (Endler, 1986). Through this process, populations become progressively more adapted to their environments.

However, natural selection can only operate on existing variation. Without phenotypic variation, populations would be unable to respond to environmental change, therefore, adaptation depends on the existence of variation within populations. The importance of standing variation is illustrated by numerous examples in nature. One of the most iconic is the peppered moth (*Biston betularia*). This species occurs in two colour morphs: the *typica* morph, characterised by whitish wings speckled with black spots, and the dark *carbonaria* morph, which is entirely black (Berry, 1990). This species rests during the day on the bark of birch trees. In this environment, the *typica* morph was better camouflaged and therefore less susceptible to predation by birds, resulting in its predominance in natural populations. However, during the Industrial Revolution in Britain, atmospheric pollution darkened tree trunks. Under these new environmental conditions, the *carbonaria* morph became better concealed from predators and rapidly increased in frequency, whereas the *typica* morph declined. Following improvements in air quality during the twentieth century, this trend reversed, and the *typica* morph once again became the most common form. This rapid evolutionary response was possible because the phenotypic variation already existed within populations before environmental conditions changed, allowing natural selection to act on standing variation (Berry, 1990).

Consequently, understanding how phenotypic variation is generated and maintained through time remains one of the central challenges of evolutionary biology. While variation is essential for adaptation, evolutionary forces can both create and erode it, raising important questions about the mechanisms responsible for its persistence in natural populations.

II. Origins and maintenance of phenotypic variation

II.1 Origin of phenotypic variation

Understanding phenotypic variation requires first identifying the processes that generate it. At the beginning of the 20th century, Johannsen created selection lines based on bean seed size and observed that size variation was the consequence of genetic factors but also of environmental influences such as the position of a bean within a pod. He consequently introduced a key distinction: the genotype, referring to all the genes of an individual, and the phenotype, all observable characteristics shaped by genotype, environment and their interactions (Johannsen, 1991). This distinction laid the foundations for our current understanding of phenotypic variation. The total phenotypic variance observed within a

population can be partitioned into three major components: genetic variance (V_G), environmental variance (V_E), and genotype-by-environment interaction variance ($V_{G\times E}$) (Orgogozo et al., 2015; Pigllucci, 1996). This relationship is summarized by the equation: $V_P = V_G + V_E + V_{GxE}$

III.1.2 Genetic sources of phenotypic variation

The contribution of genetic differences to phenotypic variation was further established by Morgan and his collaborators, who demonstrated that mutations in genes located on chromosomes could produce heritable phenotypic differences in *Drosophila melanogaster* (Morgan, 1915). Genetic variation reflects differences in DNA sequence among individuals. These genetic differences may alter gene expression or protein function, potentially generating phenotypic differences.

Mutation represents a major source of novel genetic variation because it continuously introduces new alleles into populations. Mutations range from single-nucleotide substitutions to chromosomal rearrangements, although only a subset has measurable consequences for phenotype. Point mutations, substitutions, insertions, or deletions of a single nucleotide, can occur anywhere in the genome. When they occur within coding sequences, non-synonymous mutations can alter the amino acid sequence of a protein and consequently modify its function. For example, a single point mutation in the *melanocortin-1-receptor* (*MC1R*) gene resulting in a replacement of glutamate by lysine, causes melanism in bananaquits (*Coereba flaveola*). This species occurs in two colour morphs, a yellow morph and a melanic morph, and this amino acid substitution is present in all melanic individuals (Theron et al., 2001). Interestingly, the same amino acid substitution produces a similar melanic phenotype in both mice and chickens.

Beyond point mutations, larger structural variants might also contribute to phenotypic diversity. These include chromosomal inversions, duplications, copy number variation, and the insertion of transposable elements. Chromosomal inversions, for example, have been associated with numerous adaptive traits in mosquitoes of the genus *Anopheles*, including insecticide resistance as well as behavioural and morphological variation (Ayala et al., 2014). More specifically, in *A. gambiae,* the *2Rd* and *2La* inversions are associated with resting behaviour, with higher inversion frequencies being associated with indoor resting rather than outdoors (Bryan et al., 1987). Copy number variation (CNVs), corresponding to the duplication or

deletion of genomic regions of variable size, also contributes to phenotypic variation. For instance, the dorsal hair ridge of the Rhodesian Ridgeback and Thai Ridgeback dogs is associated with the duplication of a 133-kb region on chromosome 18 containing three fibroblast growth factor genes (Salmon Hillbertz et al., 2007). Likewise, a 16-kb duplication located approximately 350 kb upstream in the regulatory region of the *HAS2* gene is associated with the characteristic skin folds of the Chinese Shar-Pei (Olsson et al., 2011). Another source of genetic variation is the insertion of transposable elements. As mentioned above, two colour morphs exist in the peppered moth (*Biston betularia*). The *carbonaria* morph is the result of the insertion of a transposable element within the first intron of the *cortex* gene, leading to increased melanisation (Hof et al., 2016). While mutation generates new genetic variants, recombination during meiosis reshuffles existing alleles into novel combinations, thereby creating additional genetic diversity within populations. In maize, for example, recombination generates new allelic combinations that contribute to variation in agronomically important traits (Pan et al., 2016).

II.1.2 Environmental sources of phenotypic variation

As stated above, genetic differences alone cannot explain all phenotypic variation. Individuals sharing the same genotype often develop distinct phenotypes when exposed to different environmental conditions. This ability of a single genotype to produce multiple phenotypes in response to environmental variation is known as phenotypic plasticity (Bradshaw, 1965). Phenotypic plasticity may allow organisms to adjust their morphology, physiology, behaviour, or life-history traits according to prevailing environmental conditions, thereby increasing their capacity to cope with environmental heterogeneity (Lynch & Gabriel, 1987). A wide variety of environmental factors can induce plastic responses. Abiotic factors such as temperature, nutrition, humidity, or photoperiod frequently influence developmental trajectories and adult phenotypes. For example, temperature determines sex in many reptiles, including all crocodilians, some lizards and many turtles (Bull, 1980). While nutritional conditions regulate caste differentiation in honeybees (*Apis mellifera*) (Weaver, 1955). In fact, early in development, female larvae are similar, and the differentiation into a queen or a worker depends on the food they receive. While future queens are fed exclusively with royal jelly throughout their development, future workers receive it for just the first three days, before transitioning to a pollen-rich diet (Weaver, 1955). Biotic factors can also induce plastic

responses. Predator presence can trigger the development of defensive structures. This phenomenon is remarkably well studied in the zooplankton genus *Daphnia* (Barry, 1999). They detect the presence of predators by a chemical signal that predators release into their environment, and *Daphnia* species respond by developing different types of defence structures. For instance, *D. carinata* grows a large crest in the presence of notonectids of the genus *Anisops* (Barry, 1994). Another environmental factor that can induce plastic responses is population density. Indeed, in the migratory locust (*Locusta migratoria*), density can influence many morphological and behavioural traits (Hamouda et al., 2011). Individuals of the solitarious morph are smaller and have a brown colouration, whereas individuals of the gregarious morph are bigger and have a yellow colouration (Hamouda et al., 2011). Plastic responses may occur during development, leading to irreversible changes in phenotype, or remain reversible throughout an individual's lifetime. Seasonal changes in coat or plumage colour observed in many vertebrates illustrate reversible plasticity, whereas caste differentiation in eusocial insects represents a developmental plastic response that becomes fixed once development is complete.

Importantly, phenotypic plasticity does not involve changes in DNA sequence. Instead, environmental cues modify patterns of gene expression through epigenetic mechanisms such as feedback loops of transcription factors, DNA methylation or histone modifications. These molecular processes enable organisms to translate environmental information into alternative phenotypes. In honeybees (*Apis mellifera*), the dimorphism between queens and workers is the consequence of multiple epigenetic marks, like DNA methylation (Elango et al., 2009; Kucharski et al., 2008), but also differences in the deposition of histone modifications. Indeed, workers have more H3K4 methylation modifications across their genome than queens, and these modifications are associated with repression of genes. Whereas, queens have a higher number of H3K27 acetylation which is associated with a higher expression of genes (Jin et al., 2023).

The relationship between environmental conditions and phenotype is commonly represented by a reaction norm, which describes the range of phenotypes produced by a single genotype across different environments. Reaction norms provide a powerful framework for quantifying phenotypic plasticity and comparing plastic responses among genotypes.

II.1.3 Genotype-by-environment sources of variation

Environmental variation does not only affect phenotypes directly but also alters modify the effects of genetic variation through genotype-by-environment interactions. A genotype that performs well in one environment may perform poorly in another, meaning that the phenotypic consequences of genetic variation depend on the environmental context in which genes are expressed. Genotype-by-environment interactions are represented by differences in reaction norms among genotypes. When reaction norms are parallel, genotypes respond similarly to environmental variation, and no G×E interaction is present. In contrast, non-parallel reaction norms indicate that genotypes differ in their plastic response. Such interactions imply that different genotypes may be favoured under different environmental conditions. For instance, in the fruit fly (*Drosophila melanogaster*), temperate and tropical subspecies genetically differ for traits such as body size, diapause or fecundity, which reflect an adaptation to ambient temperature (Lazzaro et al., 2008).

Genotype-by-environment interactions provide an important link between genetic variation and phenotypic plasticity, illustrating that the effects of genes cannot be fully understood independently of the environments in which they are expressed.

II.2 Modification of phenotypic variation

Although phenotypic variation is continuously generated through genetic and environmental processes, it is also constantly reshaped by evolutionary forces. Consequently, phenotypic variation observed within natural populations reflects a dynamic balance between processes that generate variation and those that reduce it through time. Four main evolutionary forces influence allele frequencies within populations: mutation, gene flow, genetic drift, and natural selection. These processes act simultaneously, even though their relative importance depends on ecological and demographic conditions.

As described previously, mutation represents a major source of variation by continuously introducing new alleles into populations. Genetic drift, first formalised by Wright (Wright, 1948), alters allele frequencies through random sampling rather than differences in fitness. Consequently, drift may lead to the fixation or loss of alleles independently of their selective value, an effect that becomes increasingly important as population size decreases. During the 19th century, the northern elephant seal (*Mirounga angustirostrus*) was extensively

hunted, reducing its population size to just about 20 individuals. Although populations have recovered, the extreme bottleneck they experienced, along with the resulting genetic drift and inbreeding, has drastically reduced genetic variation compared with a closely related species, the southern elephant seal (*Mirounga leonine*) (Hoelzel et al., 1993). Then, gene flow, which refers to the transfer of alleles between populations, can either increase or decrease genetic and phenotypic variation, depending on the genetic composition of the migrating group of individuals. For instance, founder effects occur when a new population is established by a small number of individuals carrying only a subset of the genetic diversity present in the source population, thereby reducing genetic variation (James, 1970). Since the new population size is small, the impact of genetic drift is stronger.

As discussed previously, when phenotypic differences are heritable and influence fitness, natural selection changes the frequency of alleles associated with those phenotypes. Alleles that increase fitness tend to become more common across generations, whereas alleles associated with lower fitness decrease in frequency. In doing so, natural selection promotes adaptation by increasing the frequency of more advantageous phenotypes within populations (Darwin, 1959). However, the consequences of natural selection for phenotypic variation depend on the selective regime acting on a trait. While some forms of selection reduce variation, others can maintain it.

Directional selection favours one extreme of the phenotypic distribution and is generally expected to reduce both genetic and phenotypic variation by increasing the frequency of alleles associated with the favoured phenotype. For instance, cichlid fishes are one of the most striking examples of evolutionary radiation. A single lake can house a hundred of species that occupy different macrohabitats and have evolved different feeding morphologies. It has been shown that directional selection on the oral jaw apparatus led to the evolution of these differences between species (Albertson et al., 2003). Under persistent strong directional selection, advantageous alleles may eventually become fixed within the population, leading to a reduction of genetic variance (Bulmer, 1971).

Similarly, stabilising selection tends to reduce phenotypic variation (Bulmer, 1976). Rather than favouring one extreme phenotype, stabilising selection favours intermediate phenotypes while selecting against individuals exhibiting extreme trait values (Schmalhausen, 1949). As a result, the population mean remains relatively constant while phenotypic variance decreases. In humans, birth weight is a classic example of stabilising selection, as low and high

birth weights are correlated with reduced survival, whereas intermediate weights are associated with higher survival in both sexes (Karn & Penrose, 1951).

In contrast, disruptive selection favours individuals expressing extreme phenotypes at both ends of the phenotypic distribution while selecting against intermediate phenotypes. This form of selection can maintain phenotypic variation within populations (Bulmer, 1976) and, under certain ecological conditions, may promote ecological diversification or even contribute to speciation. In wild populations of the African finch (*Pyrenestes ostrinus*), multiple traits of the bill, such as the lower mandible width and length, have a bimodal distribution. In this species, disruptive selection is related to the hardness of the seeds they feed on, as large morphs feed more efficiently on the hard seeds and the smaller morphs on the soft seeds (Smith, 1993).

Finally, balancing selection encompasses a variety of mechanisms that can actively maintain genetic variation within populations. Unlike directional selection, balancing selection prevents the fixation of a single advantageous allele by favouring the persistence of multiple alleles over evolutionary time. Several evolutionary processes can generate balancing selection. One of the best-known mechanisms is heterozygote advantage, whereby heterozygous individuals have higher fitness than either homozygote. As a consequence, both alleles are maintained within the population because neither can reach fixation without reducing the frequency of the advantageous heterozygous genotype. For instance, the sickle-cell allele (HbS) in humans. Although individuals homozygous for the HbS allele develop sickle-cell disease, heterozygotes exhibit increased resistance to malaria, allowing both alleles to persist in populations exposed to the parasite (Allison, 1954). Another mechanism is frequency-dependent selection, which occurs when the fitness of a phenotype depends on its frequency within the population. Under positive frequency-dependent selection, common phenotypes enjoy a selective advantage because they are common, which does not readily maintain phenotypic variation (Chouteau et al., 2016). Warning colouration and defensive mimicry are antipredator strategies known to be under positive frequency-dependent selection. Indeed, naïve predators learn to avoid coloured prey which they associate with toxicity; thus, survival increases for individuals who match a locally abundant warning signal (Chouteau et al., 2016). In contrast, under negative frequency-dependent selection, rare phenotypes have a selective advantage precisely because they are rare. As these phenotypes increase in frequency, their advantage declines, allowing alternative phenotypes to persist within the population. This dynamic can maintain substantial levels of both genetic and phenotypic variation (Fitzpatrick et al., 2007).

Understanding how these forces interact to shape phenotypic diversity is essential for explaining the remarkable diversity observed in nature and provides the foundation for investigating the evolution of traits experiencing particularly strong selective pressures. Sexual selection provides a particularly powerful framework in which to address this question because it often generates some of the strongest forms of directional selection observed in nature.

III. The case of sexually selected traits

III.1 Sexual reproduction: from isogamy to anisogamy

Sexual reproduction is characterised by biparental reproduction *via* specialised haploid cells known as gametes. In isogamous species, gametes have a similar morphology but different compatibilities, meaning that these gametes cannot fuse with all gametes from individuals of the same species (Billiard et al., 2011). Anisogamy (different gamete morphology) is thought to have evolved from this ancestral state, and may be the result of a primordial conflict over the allocation of energy to the production of gametes (Arnqvist & Rowe, 2005). This difference in gamete morphology defines the sexes of individuals in these species; the female gamete contains abundant energy reserves, necessary for embryo development, and is relatively immobile. Whereas the male gamete contains only genetic material and is highly mobile. The energy-intensive production of gametes for females means they are few, and male gametes, as a result, are more energy-efficient and numerous (Parker et al., 1972). These differences result in asymmetric costs between the sexes and are fundamental to explain differences between male and female reproductive strategies, including pre-mating behaviours (Trivers, 1972). It has also been proposed that the operational sex ratio (Emlen & Oring, 1977), that is, the ratio of sexually active males to sexually active females in a population, and the potential reproductive rate, that is, the rate at which each sex in a population could reproduce if given unlimited mates (Glutton-Brock & Vincent, 1991), also play a role in the reproductive strategies of the sexes (Kokko & Jennions, 2008). Consequently, males and females tend to have very different reproductive strategies. These differences can induce distinct responses to selective pressures and can result in phenotypic divergence between them, also known as sexual dimorphism (Kasimatis et al., 2017).

III.2 Sexual selection: conceptual framework and evolutionary role

This dimorphism is primarily fuelled by sexual selection (Andersson & Iwasa, 1996), although different mechanisms of natural selection can intervene, such as sex-specific predation pressures (Baker & Parker, 1979) or sex-specific use of ecological niches (Slatkin, 1984). Sexual selection is considered as a subset of broad-sense natural selection, which comprises all possible components of fitness (Endler, 1986). It is defined by Shuker and Kvarnemo (2021) as "any selection that arises from fitness differences associated with non-random success in the competition for access to gametes for fertilisation". Therefore, a trait evolves under sexual selection when it predictably covaries with variation in access to gametes when they are a limited resource (Shuker, 2010). It has been shown that mating is more difficult for the more abundant sex in the operational sex ratio, which also generally has a higher potential reproductive rate, resulting in a stronger sexual selection on this sex (Emlen & Oring, 1977).

III.2.1 Mate preference and the evolution of ornaments

Sexual selection acts through various mechanisms, especially through mate choice and intrasexual competition (Olito & Connallon, 2019). Mate choice is defined by the preference of one sex for some traits expressed by the other sex, resulting in non-random mating (McCullough et al., 2016). In many taxa, females choose males based on one or multiple traits, which could lead to the evolution of exaggerated structures referred to as ornaments and courtship displays that make males even more conspicuous. Female preference is thought to occur when females are predominant in a population compared to males, biasing the adult sex ratio (ASR) (Kokko & Jennions, 2008). Birds represent good examples where female choice is prevalent (Hill & McGraw, 2006). For instance, in the monogamous swallow (*Hirundo rustica*), females prefer to mate with males with a longer and more symmetrical tail, which has led to the exaggeration of the trait (Møller, 1988, 1992). One of the most striking examples of female choice occurs in birds-of-paradise from the Paradisaeidae family, which exhibit some of the most elaborated ornaments and displays (Frith et al., 1998). Males from these species indeed evolved unique courtship phenotypes, considered as a suite of traits influenced by female choice, such as plumage ornaments, behaviours and sounds used during courtship (Scholes, 2008). In other lineages, such as some guppies (*Poecilia reticulata*), females choose males based on their colouration in a negative frequency-dependent manner (Farr, 1976; Hughes et

al., 2013). Indeed, it has been shown that in laboratory populations (Hughes et al., 1999) and in wild populations (Hughes et al., 2013), females prefer to mate with males presenting a novel colour pattern. In butterflies, female choice is based on both visual and olfactory cues, the latter having a strong influence on their preference (Andersson et al., 2007). In some species, especially in fish, females choose their mates based on the nest quality, which is a proxy for the parental care they will provide to the brood. In fact, in the common goby (*Pomatoschistus microps*), females are more likely to mate with males who built a nest with a small entrance, as it increases the defence against predators and is correlated with the capacity of males to oxygenate the eggs (Jones & Reynolds, 1999). Moreover, females prefer males that already have eggs in their nest (Reynolds & Jones, 1999). Although it is not known whether the presence of eggs signals to other females or whether it enables females to reduce their energy expenditure by spending less time choosing a mate. Moreover, it can also be explained by the fact that some make the same choice as those who came before them (Reynolds & Jones, 1999).

III.2.2 Intrasexual competition and the evolution of weapons

However, in other taxonomic groups, competition between males is not mediated by mate choice. Instead, individuals of the same sex fight directly for access to the other sex or resources needed for mating. Male-male competition can lead to the evolution of exaggerated structures called weapons that will increase male mating success through dominance (Glutton-Brock & Vincent, 1991). For instance, in dung beetles (Scarabaeinae), horns evolved independently in thousands of species on at least five different body parts (Emlen, 2008). In those species, mating happens in burrows in which females lay eggs and males use their horns in tunnels to fight over reproductive access to females. Large males with horns are more successful at blocking the passage of rivals to the tunnel, and so they will have an increased fertilisation success (Emlen, 2008). Smaller males developed other strategies to have access to reproduction, like tunnelling around competing males. In cervids, males fight each other over direct access to females by using antlers, which are rigid bony cranial outgrowths (Clutton-Brock et al., 1979). Alternatively, males can fight to secure territories or other resources used by females, as in the leaf-footed cactus bug (*Narnia femorata*), in which males compete over territories on cacti used by females to lay eggs (Procter et al., 2012). Opponents display their hind legs to assess one another, and when the competition escalates, males grab each other with their hindmost legs (Procter et al., 2012). According to theory, to avoid the high cost of fighting,

weapons should be used as status badges to signal strength to males and resolve contests without fighting. However, when opponents are similar in body or weapon size, the risk of escalation increases and these traits are directly used to fight (Enquist & Leimar, 1983). Therefore, sexually selected weapons can be represented as a continuum going from weapons used in direct combat to communication signals used to threaten opponents. Moreover, the position of these traits along the continuum depends on how frequently they are used in direct fights compared to their use as signals (McCullough et al., 2016).

II.2.3 Post-mating sexual selection

The examples presented so far occurred before mating, however, sexual selection can also act after it. Polyandry creates potential for post-mating and pre-zygotic sexual selection through cryptic mate choice and sperm competition, as individuals mate with multiple partners (Parker, 1970; Thornhill, 1983). Cryptic mate choice is defined as any morphological, behavioural, or physiological mechanism that biases fertilisation toward the sperm of a specific male (Rodríguez, 2015). Sperm competition occurs when the ejaculates of two or more males compete to fertilise the eggs of a female (Wigby & Chapman, 2004). Cryptic mate choice and sperm competition are not mutually exclusive and often co-occur (Firman et al., 2017). One way for males to compete is by increasing the number of sperm cells transferred to females during a single or multiple matings, thus increasing their chance to fertilise eggs. However, females can mediate this competition and bias the number of sperm towards a given male by controlling the copulation duration (Firman et al., 2017). For instance, in the guppy (*Poecilia reticulata*), males displaying more attractive colours to females had longer copulations (Pilastro et al., 2007) and inseminated more sperm (Pilastro et al., 2004). Sperm competition and cryptic female choice are thought to have led to the evolution of sperm gigantism (Firman et al., 2017). In fact, larger sperm cells have a higher ability to displace already present sperm and to resist being displaced by shorter sperm (Lüpold et al., 2012). In *Drosophila melanogaster,* females favour larger sperm cells by storing them in longer seminal receptacles (Miller & Pitnick, 2002). Females can use differential sperm ejection as another mechanism to bias fertilisation based on male traits, such as size, age, species identity, courtship duration or social status (Firman et al., 2017). Moreover, females might also exert choice by incapacitating sperm within their reproductive tract. Males can also alleviate direct sperm competition by preventing females from remating through multiple mechanisms. For instance, they can physically guard females

pre- or post-copulation to prevent mating with other males (Rowe, 1992). They can also chemically induce a refractory period, as in *Drosophila melanogaster* (Firman et al., 2017), or obstruct female reproductive tracts with mating plugs, as in the spiders *Argiope* and *Latrodectus* genus (Schneider, 2014). In species that reproduce externally, such as sea urchins, fertilisation can be biased by the compatibility of proteins present on the surface of gametes (Firman et al., 2017).

III.2.4 The dual function of sexually selected traits

The different mechanisms of sexual selection are not mutually exclusive and can occur simultaneously within the same sex. For instance, in the dragonfly (*Libellula luctuosa*), males fight for territories where they will mate with females, and females exert both precopulatory and cryptic mate choice. In this species, wing colouration and body size are sexually dimorphic and evolve in response to the interaction of intrasexual competition and mate choice. Interestingly, the brown wing patch is influenced exclusively by mate choice, which suggests that sexual selection may act in combination or separately on the evolution of sexually dimorphic traits (Moore, 1990). Thus, many conspicuous sexually selected traits have a dual function (Berglund et al., 1996); for example, in a large number of taxa, traits considered as ornaments are also known to be displayed during intrasexual contests as status badges. Moreover, Berglund et al. (1996) proposed that ornaments could have first evolved from intrasexual competition. According to them, weapons could be considered honest signals since they are more costly to produce for individuals of low quality and are regularly tested in combat against other rivals, and members of the other sex could benefit from this information to select high-quality partners (Berglund et al., 1996). In addition, the most exaggerated weapons strongly correlate with male quality and are used for assessment during contests and rarely inflict damage on opponents (Miller et al., 2026; West-Eberhard, 1979). In contrast, species with less elaborate weapons inflict more frequent and serious damage to their opponents. The elaboration of weapons may stem from their use as signals to other rivals, suggesting an evolutionary shift from small, dangerous traits efficient for fighting towards larger, more complex ones efficient at preventing fights by signalling male quality (Miller et al., 2026). When individuals of the other sex also use these traits to choose their mate, it could lead to the evolution of ornaments. Moreover, both ornaments and weapons often have other functions besides reproduction, like leaf-footed bugs that use their legs for locomotion (Miller et al.,

2019). Additionally, colouration can also be associated with camouflage, thermoregulation or protection against UV. Therefore, the evolution of these traits is often the outcome of various selective pressures acting simultaneously and sometimes in opposing directions (Miller et al., 2026).

III.2.5 Sexual selection in females: male choice and female-female competition

Most research in the field of sexual selection has focused on males due to the parental investment theory (Kokko & Heubel, 2008), and because they generally display the most conspicuous traits. Furthermore, it has long been thought that females could not be targets of sexual selection, and that ornaments or weapons in females were only the result of a genetic correlation between sexes. However, this view has been challenged by a growing body of work demonstrating sexual selection in females through male choice and female-female competition. In many oviparous species, males preferentially mate with larger females because body size is often positively associated with fecundity, thereby increasing their expected reproductive success (Bonduriansky, 2001; Dosen & Montgomerie, 2004; Reading & Backwell, 2007). In the glow-worm (*Lampyris noctiluca*), females glow to attract males and their brightness is correlated with fecundity. Therefore, males are more likely to choose females with a brighter abdomen (Hopkins et al., 2015). Female-female competition is also more and more documented. Beyond mate choice and intrasexual competition, sexual selection can also arise through scramble competition, where reproductive success depends on the ability to locate and access mates rapidly. Such mechanisms have been documented across a wide range of taxa, including plants. Together, these examples illustrate that sexual selection is neither restricted to males nor limited to a single mechanism. Instead, it operates across sexes and taxa, generating a remarkable diversity of sexually selected traits that differ in their morphology and function.

III.2.6 Sexual conflict

As mentioned above, males and females have dissimilar reproductive strategies. As a consequence, their evolutionary interests do not necessarily coincide, meaning that a trait beneficial for one sex can be costly to the other (Kokko & Jennions, 2014). This process, known as sexual conflict, is widespread (Chapman et al., 2003) and involves traits shared by both sexes and/or occurs when the optimal outcomes of an interaction between males and females cannot be achieved simultaneously (Arnqvist & Rowe, 2005). Therefore, every aspect of reproduction, from mate searching to parental care, can be a source of conflict, and it is likely to be present whenever there is sexual reproduction (Kokko & Jennions, 2014). Many traits may be affected, such as primary sexual traits (Brennan & Prum, 2015), secondary traits (Arnqvist & Rowe, 2002b), or behavioural traits (Schneider, 2014). Thus, even if sexual conflict can extend beyond mating and access to the opposite sex's gametes, sexual selection is one of the major sources of sexual conflict (Kokko & Jennions, 2014).

III.2.7 The evolution of mate preference and ornaments

Mate choice is an evolutionary puzzle that biologists have been trying to explain for decades. Indeed, how could a preference for an apparently detrimental feature have possibly evolved among females in the first place? Sensory drive is one of the most prominent hypotheses proposed to explain the origin of the preference-display coevolution between sexes (Payne & Pagel, 2001). This hypothesis predicts that both communication signals and perceptual systems will adapt to environmental conditions. Sensory systems are shaped by environmental conditions, thus leading to potential biases. These biases could lead to mating biases and mate preference if females inadvertently respond to a male trait that has an impact on fitness (Kokko et al., 2003). For instance, female guppies are sensitive to orange-coloured food, which may explain their preference for males with large orange spots (Rodd et al., 2002). Once preference is initiated, different models have been proposed to elucidate the coevolution between mate preference and ornaments. For mate preference to evolve, females should benefit from their choice either directly or indirectly (Kokko et al., 2003). Firstly, females should benefit directly from their choice when they receive “material” advantages, such as superior resources, better parental care or even reduction of reproductive costs (Haywood, 1993). Fisher is one of the first to have suggested indirect benefits as a mechanism underlying mate choice

(Fisher, 1930). The offspring of successful males and of females that mate preferentially with them will inherit both alleles encoding for the preferred traits and alleles linked to the mating preference, thereby increasing their own mating success. Thus, loci for trait expression and for preference become linked and evolve together. In 1981, Lande took into account the polygenicity of preference and trait expression and demonstrated the genetic correlation hypothesised by Fisher (Lande, 1981). The genetic correlation will generate a positive feedback loop leading to the exaggeration of traits until halted by natural selection, which is called Fisher's runaway process (Fisher, 1930). It is closely associated with the "good genes" and the "sexy sons" models. The "good genes" model assumes that male ornaments are honest signals of their quality, and that it should therefore benefit females, as their offspring will have increased viability (Chandler et al., 2013; Møller & Alatalo, 1999). The "sexy sons" model predicts that sons of males with the most elaborated ornaments will have a higher mating success, thus benefiting females. Indirect benefits are supposed to be consistently present, because even when males provide direct benefits, their ability to produce attractive signals varies (Kokko et al., 2003).

III.2.8 The evolution of weapons

Whilst mate choice fascinated and raised many questions, the origins of intrasexual competition and the evolution of weapons were set aside, as it was thought that almost everything had already been elucidated. This bias in the literature has led to the misconception that the term "ornaments" could be used to refer to all sexually selected traits (McCullough et al., 2016). Not all species that engage in intrasexual competition evolve exaggerated weapons. Species that do evolve exaggerated structures have in common that individuals of the fighting sex compete for critical resources that are patchily distributed, such that one or a few males can defend them at the same time, thus biasing access to gametes (Emlen & Oring, 1977). Hence, the ecology and behaviours of species could play a role in the evolution of exaggerated traits (Emlen, 2008). Beyond determining the intensity of competition, interactions among rivals themselves may also influence the evolution of weapons. As mentioned above, opponents assess one another during contests and decide whether to escalate contests based on their probability of winning or losing the fight (McCullough et al., 2016). Thus, the social context influences how individuals display their weapons. Building on this idea, Moore et al., (2022) showed that these socially mediated interactions could generate a positive feedback process

analogous to Fisherian runaway selection. Specifically, their quantitative genetic model predicts runaway elaboration of weapons when the costs imposed on rivals increase with body size, making weapons function as honest signals of competitive ability, and males consequently adjust their aggressive behaviour. Conversely, alternative modelling approaches suggest that exaggeration can also arise in the absence of runaway dynamics, through direct selection on fighting ability and signal honesty, highlighting that the mechanisms underlying weapon exaggeration have yet to be elucidated (Dashtbali et al., 2024).

III.2.9 Maintaining genetic variation under strong sexual selection

Regardless of the mechanism responsible for trait exaggeration, sexually selected traits are expected to be under strong directional selection. Such persistent selection should erode genetic variance and, ultimately, reduce phenotypic variation (Fisher, 1930). However, in natural populations variation is commonly observed to be equal to or even higher than non-sexually selected traits (Kotiaho, 2001; Pomiankowski & Møller, 1995). This apparent contradiction, originally formulated in the context of mate choice, is known as the "lek paradox" (Kirkpatrick & Ryan, 1991). Nevertheless, similar questions arise for exaggerated weapons as they are also subject to strong and persistent sexual selection through male-male competition. The persistence of genetic variation despite strong and directional sexual selection has therefore been a central question in evolutionary biology. Several non-mutually exclusive mechanisms have been proposed to explain how variation in sexually selected traits can be maintained, such as mutation biases, condition dependence, environmental heterogeneity, sexually antagonistic effects, and social interactions. Most of these models were developed within the context of the "lek paradox"; nevertheless, these mechanisms may also contribute to maintaining genetic variation in weapons. One of the earliest explanations proposed that mutation-selection balance could maintain genetic variation despite directional selection. Under this view, deleterious mutations continuously reintroduce variation that is subsequently removed by selection (Pomiankowski & Møller, 1995). However, mutation alone may not fully account for the amount of variation observed in many sexually selected traits, leading to the development of alternative hypotheses. It has been demonstrated that traits subject to sexual selection are highly condition-dependent, which means that their growth tends to be more sensitive to the nutritional or physiological conditions of individuals, contrary to non-sexually selected traits (Andersson & Iwasa, 1996). For instance, in adult male rhinoceros beetles (*Trypoxylus dichotomus*), the

size of the horn is highly variable, and it has been demonstrated that it is more sensitive to larval nutrition than other parts of the body (Emlen et al., 2012). Building on this, an alternative model developed by Rowe and Houle (Rowe & Houle, 1997) suggests that sexually selected traits may have captured genetic variation that modulates an individual's condition. As trait expression became associated with condition, which is polygenic, sexually selected traits could capture genome-wide variation through condition-dependent expression, thereby maintaining high additive genetic variance. Miller & Moore (2007) intended to extend the "genic capture" of Rowe and Houle, which considered direct additive advantages, by adding the potential influence of indirect genetic effects. Indeed, parental behaviours such as habitat selection and the ability to provide resources often have a genetic origin and influence the condition, therefore trait expression in offspring. Thus, offspring will also receive "good genes" in the form of effective parental characteristics. However, variation may also persist because selection itself varies across contexts. Genetic variation might be affected by stressful environmental conditions but also by temporal and spatial fluctuations of the environment (Ingleby et al., 2010). Genotype-by-environment interactions may help to maintain sufficient genetic variation in sexually selected traits when there is high gene flow between individuals from different environments (Kokko & Heubel, 2008). Nonetheless, these interactions may also disrupt signal honesty, thereby weakening selection for female preferences. Further studies are needed to investigate in greater detail the role of the genotype-by-environment interaction in resolving the "lek paradox". Finally, sexually antagonistic selection is also hypothesised to prevent the fixation of alleles. Indeed, selection acting on variants that have opposing effects on male and female fitness is likely to contribute to the maintenance of high levels of genetic variance to balance fitness between the sexes (Chippindale et al., 2001). Models confirm that intralocus sexual conflict can be a major source of balancing selection that maintains polymorphism (Connallon & Clark, 2014), a finding also supported by a recent empirical study (Zwoinska et al., 2026). However, it remains difficult to identify signatures of balancing selection in natural populations.

Despite these theoretical advances, empirical evidence supporting these mechanisms remains limited. A better understanding of sexually selected trait genetic architecture could help investigate the mechanisms resolving the "lek paradox".

IV. Microvelia longipes as a model to study mechanisms underlying phenotypic variation in an exaggerated sexually selected trait

Gerromorpha (order Hemiptera, suborder Heteroptera) are semi-aquatic insects that have emerged as a powerful model for investigating the evolutionary processes underlying biodiversity. Their colonisation of the water–air interface opened novel ecological niches and was accompanied by the evolution of numerous morphological, behavioural, physiological, and developmental adaptations to this unique environment. For instance, all Gerromorpha species have longer legs with higher bristle density. They are thought to increase their floatability on water (Andersen, 1982). This remarkable evolutionary diversification has made Gerromorpha an important model system for studying a broad range of questions in ecology, evolution, development and biomechanics. In particular, several species have become key models for investigating sexual selection and sexual conflict. Species of the genus *Gerris*, among others, have been extensively studied to understand the evolution of mating strategies and sexually antagonistic coevolution (Arnqvist & Rowe, 2002a). More broadly, the diversity of mating systems and sexually selected traits found across Gerromorpha makes this infraorder particularly well suited for investigating how ecological and evolutionary processes interact to generate and maintain phenotypic diversity.

Among Gerromorpha, the water strider *Microvelia longipes* provides a particularly powerful model for investigating the evolution and maintenance of phenotypic variation under sexual selection. This species lives in temporary rain-filled puddles in tropical regions of South America (Andersen, 1982). Males guard small floating objects which females use as egg-laying sites. They signal to attract females to these sites and fight against other males using their rear legs over these sites (Toubiana & Khila, 2019). Previous studies have shown that males with longer rear legs are more likely to win contests. Thus, they secure more matings and sire more offspring than their shorter-legged competitors (Toubiana & Khila, 2019). Consequently, male reproductive success is largely determined by rear leg length, which has led to an exaggeration of this trait.

Despite this intense selection, rear leg length exhibits remarkable phenotypic variation within natural populations (Fig. 1).

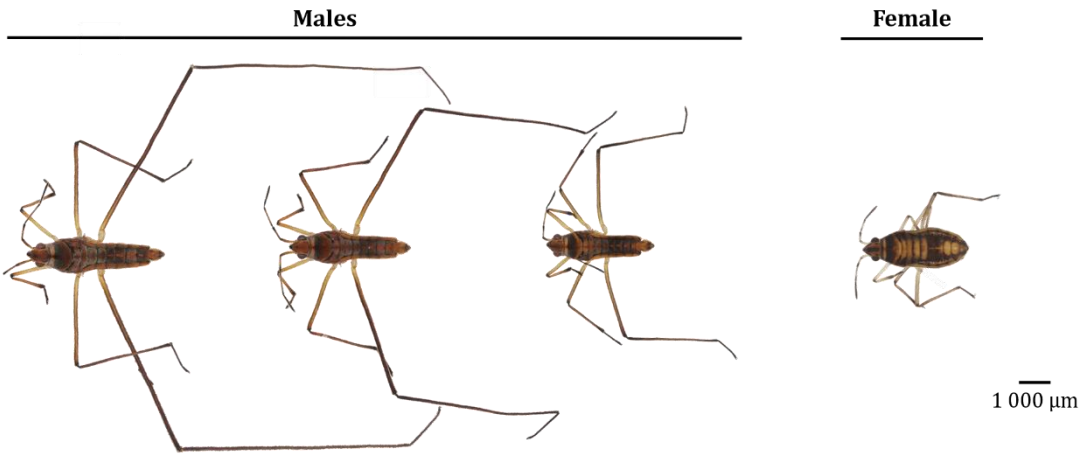

Figure 1. Phenotypic variation in *M. longipes*

This extensive variation is maintained despite the expectation that persistent directional selection should progressively reduce genetic and phenotypic diversity. *Microvelia longipes* therefore represents an ideal system for investigating one of the central questions introduced in this thesis: how can substantial phenotypic variation persist in traits experiencing strong and sustained directional selection?

Previous work has demonstrated that variation in rear leg length arises from the combined effects of genetic factors and environmental conditions. Inbreeding experiments involving sib-sib crosses, with selection favouring small or large males, have revealed a genetic contribution to both rear leg length and body size. Furthermore, differential nutrition in nymphs from one of the inbred lines revealed strong condition dependence for both traits (Toubiana & Khila, 2019). Together, these findings suggest that both genetic architecture and phenotypic plasticity contribute to the remarkable diversity observed in this trait. However, how these processes shape the expression and persistence of phenotypic variation under sexual selection remains poorly understood. In particular, the respective roles of standing genetic variation and environmentally induced variation in generating phenotypic diversity, as well as the ecological consequences of this variation, remain to be fully clarified. Addressing these questions is essential for understanding how exaggerated sexually selected traits retain substantial variation despite strong directional selection.

This thesis addresses these questions by focusing on two complementary aspects of phenotypic variation in *Microvelia longipes*. First, it examines the ecological consequences of phenotypic variation by testing whether exaggerated rear legs entail viability costs that could

contribute to the maintenance of variation. Second, it investigates how standing genetic variation contributes to phenotypic variation and how prolonged directional selection shapes both phenotypic and genomic diversity through experimental evolution and population genomic approaches. More specifically, this work aims to determine whether exaggerated weapons impose survival costs, how rear leg length responds to experimental selection, and how genomic diversity changes following experimental evolution. Together, these complementary approaches provide an integrative framework for understanding how genetic variation contributes to phenotypic variation and how ecological processes may influence its maintenance in an exaggerated sexually selected trait.

Chapter 1

Sexual selection and viability trade-offs: testing starvation resistance costs of weapon exaggeration in *Microvelia longipes* males

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Abstract

Sexual selection often drives the evolution of exaggerated traits, but their persistence in populations depends on reproductive benefits outweighing viability costs. In the semi-aquatic water strider *Microvellia longipes*, males use disproportionately long legs as weapons in intrasexual contests for access to floating oviposition sites. While longer legs confer advantages in male-male competition, the maintenance of high phenotypic variation in leg length in natural populations suggests potential trade-offs. Here, we test whether exaggerated rear legs incur survival costs by measuring starvation resistance in males and females under food deprivation. We conducted two experiments: (1) isolated individuals (87 males, 50 females) to assess intrinsic costs and (2) grouped males under low (abundant oviposition sites) or high (limited sites) competition (3 replicates, 40 males + 40 females per replicate) to evaluate context-dependent costs. Females survived significantly longer than males, indicating strong sex differences in starvation resistance. In males, body size positively influenced survival, but relative rear leg size had no detectable effect in either isolation of competitive contexts. Competition intensity also did not affect male survival. These results suggest that exaggerated rear leg weapons in M. longipes do not entail direct adult survival costs, at least under laboratory conditions. Our findings underscore the need to investigate developmental costs or ecological trade-offs to explain the persistence of phenotypic variation in weapon size.

Résumé

La sélection sexuelle favorise souvent l'évolution de caractères exagérés, mais leur maintien dans les populations dépend d'un équilibre entre les bénéfices reproducteurs qu'ils procurent et les coûts qu'ils engendrent pour la survie. Chez la punaise semi-aquatique *Microvelia longipes*, les mâles utilisent des pattes postérieures disproportionnellement longues comme armes lors des compétitions intrasexuelles pour l'accès aux sites de ponte flottants. Bien que des pattes plus longues confèrent un avantage lors des combats entre mâles, le maintien d'une forte variation phénotypique de la longueur des pattes dans les populations naturelles suggère l'existence de compromis évolutifs. Dans cette étude, nous avons testé si ces armes exagérées entraînent un coût de survie en mesurant la résistance au jeûne de mâles et de femelles soumis à une privation de nourriture. Deux expériences ont été réalisées : (1) des individus isolés (87 mâles et 50 femelles) afin d'évaluer les coûts intrinsèques liés au développement des armes, et (2) des groupes de mâles soumis à une faible intensité de compétition (nombreux sites de ponte disponibles) ou à une forte intensité de compétition (nombre limité de sites de ponte), avec trois réplicats par condition (40 mâles et 40 femelles par réplicat), afin d'évaluer d'éventuels coûts dépendant du contexte social. Les femelles ont survécu significativement plus longtemps que les mâles, révélant une forte différence entre les sexes dans la résistance au jeûne. Chez les mâles, la taille corporelle influençait positivement la survie, tandis que la taille relative des pattes postérieures n'avait aucun effet détectable, aussi bien chez les individus isolés que dans les conditions de compétition. De plus, l'intensité de la compétition n'affectait pas la survie des mâles. Ces résultats indiquent que les armes exagérées de *M. longipes* n'entraînent pas de coût direct sur la survie des adultes, du moins dans les conditions expérimentales utilisées. Ils soulignent néanmoins la nécessité d'explorer d'autres types de compromis, notamment les coûts liés au développement ou les compromis écologiques, afin de mieux comprendre les mécanismes responsables du maintien de la variation phénotypique de la taille des armes.

Introduction

Sexual selection can drive the evolution of exaggerated morphological structures characterised by disproportionate growth compared to body size (Shingleton & Frankino, 2013). Theory predicts that positive allometry evolves when the net advantages of increasing trait size is higher than the associated viability costs for larger individuals than for smaller ones (Bonduriansky, 2007). Consequently, sexually selected traits often become disproportionately elaborated in the largest males. The enlarged claws of fiddler crabs (Christy et al., 2002) and the mandibles of stag beetles (Gotoh et al., 2014) are famous examples of increased success during male-male competition for larger males. To better understand the development and maintenance of these exaggerated traits, evolutionary biologists have been trying to evaluate the viability costs associated with these traits. Exaggerated traits may impair locomotor performance, increasing predation risk (Oufiero & Garland, 2007). For instance, in the swordtail fish *Xiphophorus montezumae,* longer swords in males increase energy expenditure during routine and courtship swimming (Basolo & Alcaraz, 2003). Costs might also result from allocation trade-offs; as individuals possess finite energetic resources (Husak et al., 2016), investment in the production or maintenance of sexually selected traits may reduce the resources available for other functions, such as muscle development or spermatogenesis (Cavender et al., 2021; Simmons & Emlen, 2006). Beyond these developmental trade-offs, exaggerated weapons can elevate resting metabolic rate, potentially reducing survival under resource limitation. For example, males bearing larger weapons have been shown to exhibit higher resting metabolic rates in the leaf-footed bug *Leptoscelis tricolor* (Somjee et al., 2018). However, many species carrying exaggerated traits have evolved compensatory mechanisms that offset the negative performance incurred due to wielding the exaggerated sexually selected traits (Møller, 1996; Oufiero & Garland, 2007). In the rhinoceros beetle *Trypoxylus dichotomus,* the length of the horn has no impact on its flying ability (speed or endurance), as it seems that males have larger wings and flight muscles (McCullough et al., 2012).

Here, we test whether males of *Microvelia longipes* incur any survival costs associated with carrying exaggerated rear legs. *M. longipes* is a semi-aquatic heteropteran insect found in small temporary puddles filled with rainwater in tropical regions of South America (Andersen, 1982; Toubiana & Khila, 2019). Females use debris of twigs and dead leaves floating on the surface of water as egg-laying sites (Toubiana & Khila, 2019). Males guard these oviposition sites, signal to attract females, and fight using their rear legs as weapons to fend off competitors (Toubiana & Khila, 2019). Males with long rear legs are favoured by intrasexual competition,

which has led to the evolution of exaggerated leg length (Toubiana et al., 2021; Toubiana & Khila, 2019). While exaggerated rear leg length is favoured by sexual selection through domination of egg-laying sites, the large variation of this trait in natural populations remains unexplained. This suggests that costs, such as reduced starvation resistance, may limit the evolution of extreme leg lengths.

In this study, we will test the hypothesis that males with longer legs will have lower starvation resistance due to increased energetic expenditure demands associated with weapon maintenance and use in contests to dominate egg-laying sites. We therefore predict a negative correlation between relative leg size and resistance to starvation, particularly in competitive environments.

Materials & Methods

Study organism:

Water striders are heteropteran hemimetabolous insects from the superfamily Gerromorpha (Andersen, 1982). Species in this group undergo an incomplete metamorphosis, which means that the larvae, called nymphs, are similar to the adults. They go through 5 nymphal instars before metamorphosis (Andersen, 1982). In *Microvelia longipes*, the focal species of the thesis, there is a burst of growth of male exaggerated legs at the end of the postembryonic development during the 5th instar, and legs reach their final size in the adults (Toubiana et al., 2021). The current population of *Microvelia longipes* was established in 2013 from individuals collected in French Guiana and has been reared in the laboratory ever since. Animals were fed daily *ad libitum* on frozen crickets, and they were reared in water tanks in a room maintained at 28°C with a humidity between 40-50% under an artificial photoperiod of 16 hours. Males and females were kept in the same water tanks to mate freely. Females laid eggs on Styrofoam floaters, with tissue on them to maintain humidity, which were removed every 5 days and put into new water tanks to let the eggs hatch and to decrease nymph cannibalism.

Resistance to starvation experiments

Male rear legs are used as weapons during intrasexual competition to fend off competitors (Toubiana & Khila, 2019). Males with long rear pair of legs are favoured by intrasexual competition, which has led to the evolution of exaggerated leg length (Toubiana et

al., 2021; Toubiana & Khila, 2019). To investigate potential adult costs associated with weapon exaggeration, we measured starvation resistance, i.e. the time elapsed between the beginning of the experiment and death for each individual in the absence of food. Thus, we predicted a negative correlation between leg size and starvation resistance because large males require more energy to fight and move. Two complementary experimental designs were implemented. In the first, individuals were isolated to determine the potential costs associated with rear leg length independently of social interactions. Starvation resistance was also measured in females to assess sex differences in resistance to food deprivation. In the second, individuals were maintained in groups under food deprivation, creating a context in which both starvation and male-male competition could affect survival. Under these conditions, we hypothesised that males with the largest rear legs would experience higher mortality because they experience higher energy expenditure due to contests than males with smaller weapons.

- Experiment 1: Isolated individuals:

During nymphal development, individuals were fed a standard diet (frozen crickets *ad libitum* every day). At the 5th instar, males and females were randomly selected and then separated to standardise the age of adults and ensure that the individuals were virgins, given that reproduction involves an energy expenditure. When an individual reached the adult stage, it was isolated in a new water tank (5 cm x 5 cm) without food. Mortality was checked three times a day (9 am, 1.30 pm, 6 pm) until all the individuals had died. The experiment was carried out on 87 males and 50 females.

- Experiment 2: Grouped individuals:

Two treatments were set up, each with three biological replicates. Each replicate consisted of 40 males and 40 females, placed in a tank filled with water but without food, and the survival of the animals in this environment was monitored until all individuals had died. To standardise the age and to ensure the virginity of animals, they were randomly selected at the 5th instar and separated from the rest of the population. Once they had reached adulthood, they were selected to form a replicate (a margin of three days was required to ensure a sufficient number of individuals). In the control condition, the intensity of intrasexual competition was reduced by making the resource for which males are fighting over abundant, so multiple large floaters were added (8 cm x 1 cm), allowing several males to occupy a single floater. This

treatment reduces competition between males (Emlen, 2001). Whereas in the other condition, the intensity of competition was increased by reducing the size of the floaters available (1 cm x 0.25 cm). They were small enough for a single male to dominate, and their number was limited (1 floater per 5 males). In this condition, a floater was removed as soon as 5 males died in order to maintain a high intensity of competition. In each condition, the males were starved, meaning that the males were not fed during the whole duration of the experiment. The females were isolated each night and fed separately from the males so that they would have sufficient energy for reproduction. It has been shown in certain species of Gerromorpha that hunger has a negative impact on females' motivation to mate (Rowe et al., 1994). Mortality was checked three times a day.

Phenotyping

When an individual died, its thorax and abdomen were measured as a proxy for body size. Tibias of the rear leg were also measured using the Keyence VHX 700 macroscope, and then conserved in 90% ethanol. The length of the tibia of the rear leg was used as a proxy for the total rear leg length.

Statistical analysis

- Experiment 1: Isolated individuals:

All individuals for which the time of death was uncertain were excluded from the statistical analysis. A total of 48 males and 36 females were retained for the analysis. Survival analyses were performed using Cox proportional hazards models implemented in the R package *survival*, and as all individuals eventually died during the experiment, all observations were treated as events (status = 1). To investigate the effect of relative leg size on male starvation resistance, a body size-corrected measure of leg size was first obtained as the residuals from a linear regression of log-transformed tibia length on log-transformed body size. The effect of relative leg size on survival was then tested using a Cox proportional hazards model. Then, the effect of body size on male survival was analysed using Cox proportional hazards models including log-transformed body size as a continuous predictor. The same models were used for females. Sex differences in starvation resistance were assessed using a Cox proportional

hazards model including sex as a fixed effect. For all models, the hazard ratios and their 95% confidence intervals were calculated from model coefficients. In addition, the proportional hazards assumption was evaluated using Schoenfeld residual tests (Clark et al., 2003). Relative leg size and body size were standardised (z-transformed) to facilitate interpretation of the graphs.

- Experiment 2: Grouped individuals:

As individuals were maintained in replicate populations, survival analyses were conducted using mixed-effects Cox proportional hazards models (*coxme* package) to account for the non-independence of individuals. Differences in survival between experimental conditions were tested by including condition as a fixed effect and replicate population as a random intercept. Then, to determine whether the relationship between relative leg size and survival differed between experimental conditions, mixed-effects Cox models were fitted with the experimental condition and relative leg size as fixed effects and replicate population as a random intercept. Relative leg size was calculated similarly to the previous experiment. The effect of body size was calculated with the same model. For all models, the hazard ratios and their 95% confidence intervals were calculated from model coefficients. In addition, the proportional hazards assumption was evaluated using Schoenfeld residual tests. Likelihood ratio tests were used to compare nested models and assess the significance of interaction terms. To facilitate the visualisation of data, graphs were constructed using predictions from the coxph function from the *survival* package.

Results

Females show higher starvation resistance than males

Starvation resistance differed markedly between the sexes, as females survived significantly longer under food deprivation than males (Cox proportional hazards model: HR = 2.70, 95% CI = 1.70–4.27, $p < 0.001$; Fig. 1). Overall, males had a mortality risk 2.7 times higher than females throughout the experiment (Fig. 1).

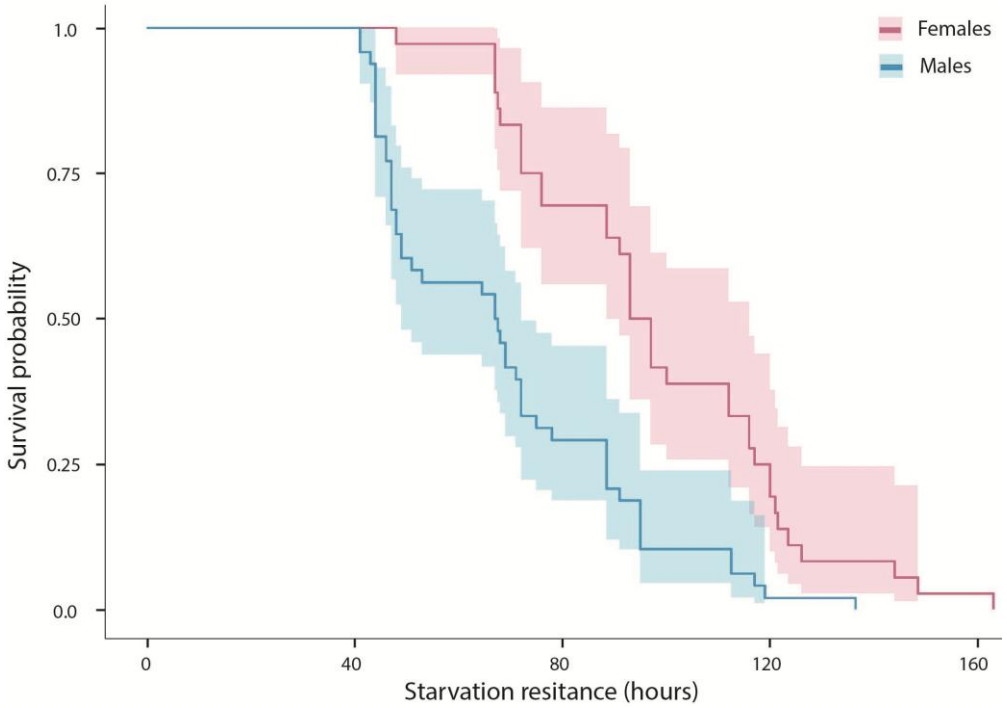

Figure 1. Predicted survival curves for 87 males and 50 females under starvation conditions. Curves were obtained from a Cox proportional hazards model fitted with sex as a predictor. Shaded areas represent 95% confidence intervals.

Body size or leg length does not influence starvation resistance

We then tested whether morphological variation explained starvation resistance within each sex. In males, neither relative rear leg size nor body size significantly impacted survival. Relative leg size, estimated as the residuals from the allometric relationship between tibia length and body size, was not associated with mortality risk (HR = 1.04, 95% CI = 0.77–1.41, p = 0.80; Fig. 2A). Likewise, body size had no detectable effect on starvation resistance (HR = 0.89, 95% CI = 0.65–1.22, p = 0.47; Fig. 2B).

In females, relative leg size also did not predict starvation resistance (HR = 1.04, 95% CI = 0.77–1.41, p = 0.80; Fig. 2C). In contrast, larger females tended to survive longer under food deprivation, although the effect was only marginally significant (HR = 0.70, 95% CI = 0.48–1.02, p = 0.064; Fig. 2D).

Together, these results indicate that starvation resistance was strongly sex-dependent, with females exhibiting greater resistance than males. However, variation in relative leg size did not explain starvation resistance in either sex, and body size only showed a weak tendency to increase starvation resistance in females.

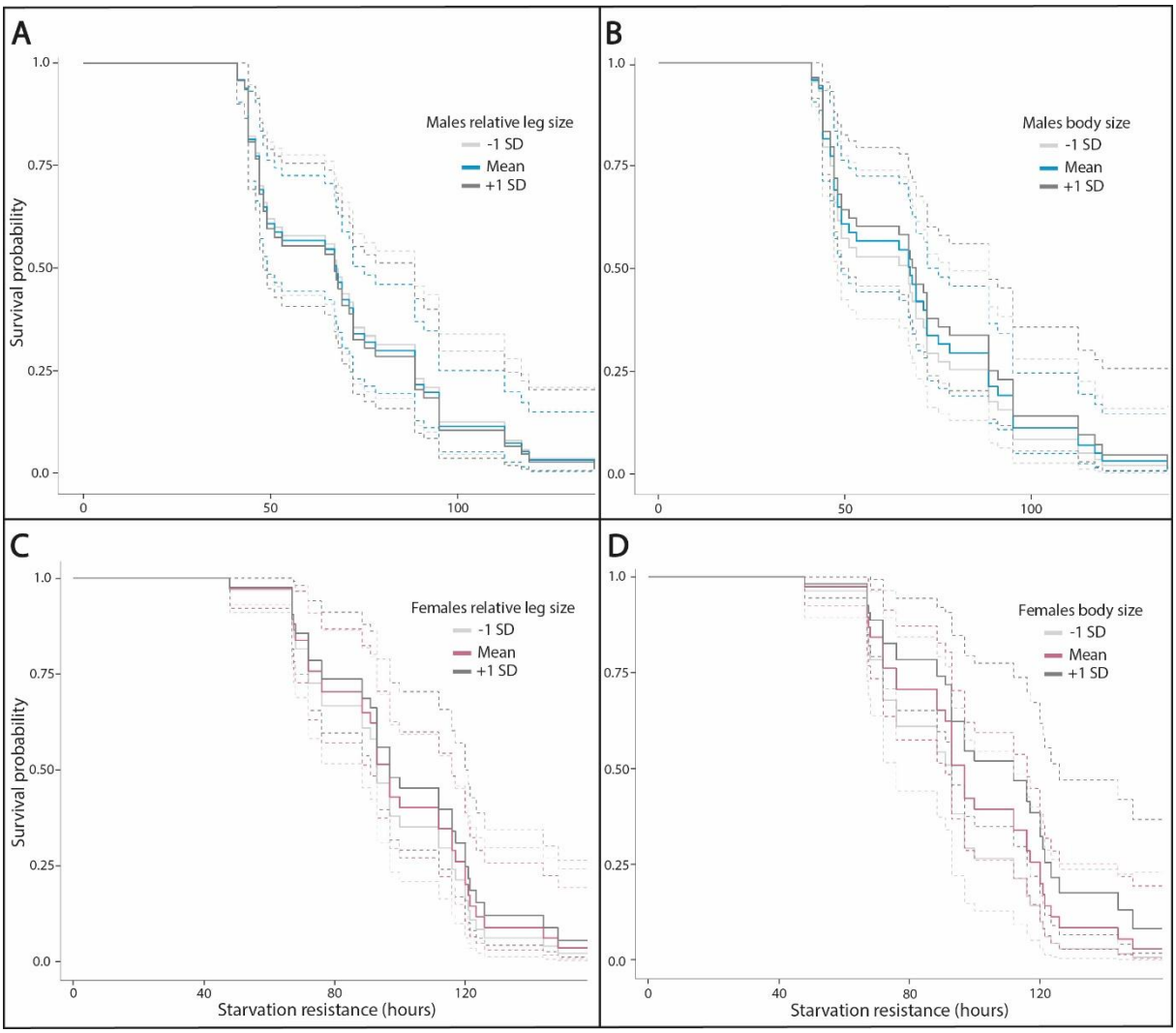

Figure 2. Predicted survival curves under starvation for individuals differing in morphological traits. Curves were obtained from a Cox proportional hazards model. Morphological traits were standardised prior to analysis (mean = 0, SD = 1), and curves correspond to individuals with morphological values one standard deviation below the population mean (−1 SD), equal to the population mean (Mean), and one standard deviation above the population mean (+1 SD). Dashed lines represent 95% confidence intervals. (A) Predicted curves for males depending on their relative leg length. (B) Predicted curves for males depending on their relative body size. (C) Predicted curves for females depending on their relative leg length. (D) Predicted curves for females depending on their relative body size.

The intensity of competition does not influence males' starvation resistance

Starvation resistance did not differ between experimental conditions. The mixed-effects Cox proportional hazards model revealed no effect of treatment on mortality risk (HR = 0.96, p = 0.92), indicating that males maintained under the higher male-male competition did not have reduced survival compared to control males (Fig. 3). Variation within conditions was

higher than the variation between them, as indicated by the random effect included in all models (Supplementary Fig. S1).

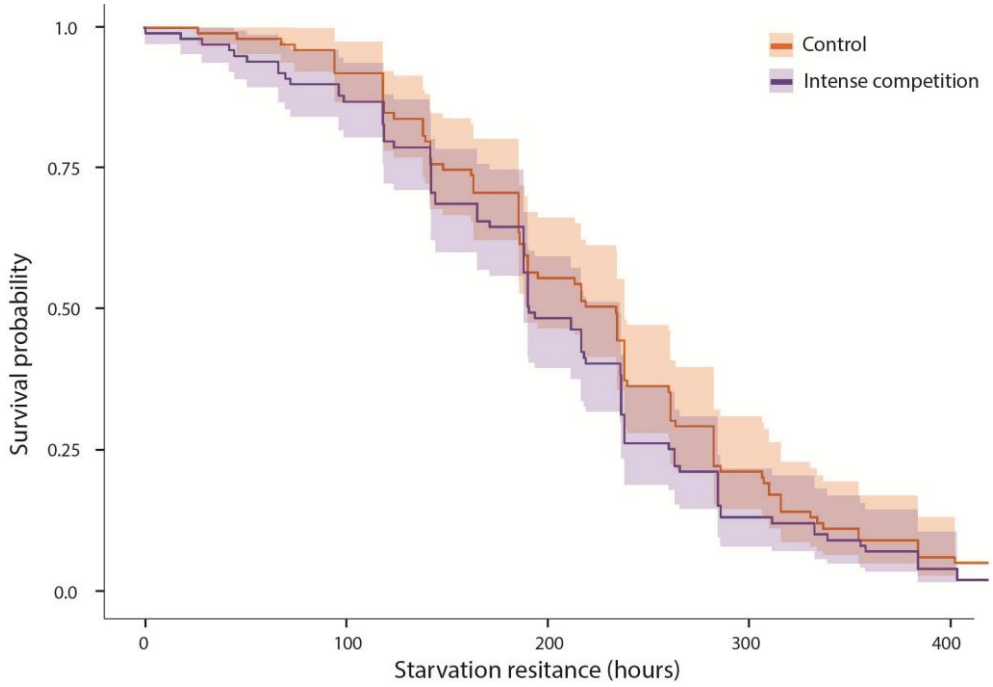

Figure 3. Predicted survival curves for males from the control and the intense competition conditions. Curves were obtained from a Cox proportional hazards model fitted with sex as a predictor. Shaded areas represent 95% confidence intervals

Larger males withstand starvation better regardless of the intensity of competition

Body size, however, significantly influenced starvation resistance. Larger males showed a lower mortality risk under food deprivation both in high and low competition treatments (HR = 0.66, 95% CI=0.56-0.78, $p < 0.001$; Fig. 4A, 4B). This effect was consistent across experimental conditions, as the interaction between body size and treatment was not significant ($p = 0.30$). Thus, larger males survived longer regardless of the intensity of competition.

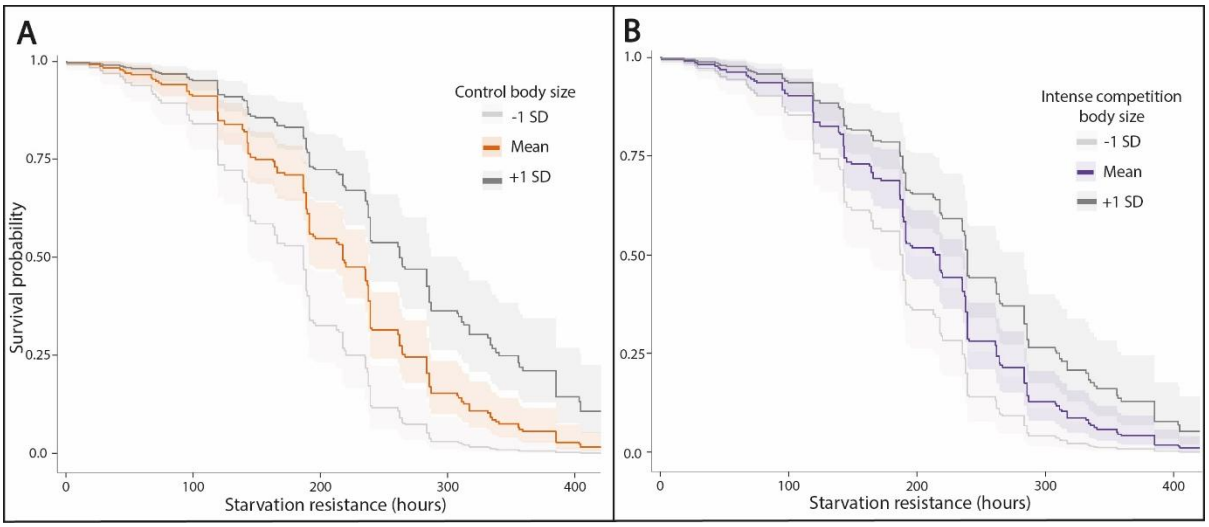

Figure 4. Predicted survival curves for males from the control and the intense competition conditions. Curves were obtained from a Cox proportional hazards model. Body size was standardised prior to analysis (mean = 0, SD = 1), and curves correspond to individuals with body size values one standard deviation below the population mean (−1 SD), equal to the population mean (Mean), and one standard deviation above the population mean (+1 SD). Shaded areas represent 95% confidence intervals. (A) Predicted curves for males from the control condition. (B) Predicted curves for males under intense competition.

In contrast, relative rear leg size did not affect starvation resistance (HR = 1.04, 95% CI=0.84-1.28, p = 0.64) nor its interaction with treatment (p = 0.26) significantly explain variation in survival (Supplementary Fig. S2).

Overall, starvation resistance in grouped males was primarily associated with body size rather than leg size or experimental condition. Larger males consistently survived longer under food deprivation, whereas relative weapon exaggeration did not predict mortality risk.

Discussion

Contrary to our predictions, males bearing exaggerated rear legs did not exhibit lower starvation resistance, either in isolation or under competitive conditions. In contrast, body size consistently predicted survival under starvation, whereas females survived longer than males. We first discuss the higher starvation resistance observed in females before considering the evolutionary implications of the apparent absence of detectable costs associated with exaggerated rear legs in males.

Overall, males survived substantially longer under starvation when maintained in groups (median survival time ≈ 200 hours) than when isolated (median survival time ≈ 70 hours). *M. longipes* is a gregarious species whose individuals live together throughout their life cycle. Social isolation is known to induce physiological stress that can impact immune responses (Scharf et al., 2021), shorten lifespan in ants (Koto et al., 2023) and can influence sleeping and feeding behaviours in *Drosophila melanogaster* (Li et al., 2021). Thus, social isolation may have represented an additional stressor that reduced starvation resistance beyond the effects of food deprivation alone. However, *M. longipes* is both cannibalistic and scavenging; these behaviours may also have contributed to the longer survival observed in groups, even though we tried to limit this by removing all dead individuals when we checked mortality.

Moreover, in the social experiment, larger males resisted starvation better. Indeed, larger individuals potentially possess greater resources because storage tissues generally increase with body size (Reim et al., 2006). Although larger bodies require greater maintenance costs (Gergs & Jager, 2014), energy expenditure has a negative relationship with body size, allowing larger individuals to expend relatively less energy. Larger individuals are also hypothesised to store reserves and mobilise energy in a different manner than smaller ones (Gergs & Jager, 2014).

Interestingly, weapon size did not translate into a detectable survival cost associated with exaggerated rear legs. This finding is consistent with a growing body of literature suggesting that the viability costs of sexually selected traits are often lower than initially expected (Thavarajah et al., 2016). The costs associated with exaggerated weapons are notoriously difficult to quantify because compensatory mechanisms may partially or entirely offset them (McCullough et al., 2012). Building on this hypothesis, the rear legs of *M. longipes* also serve locomotor functions, so natural selection may constrain their morphology and favour compensatory adaptations that reduce the mechanical or energetic costs associated with weapon exaggeration. In the leaf-footed bug *Leptoscelis tricolor*, males with larger hind legs exhibit higher resting metabolic rates, although the increase is smaller than expected (Somjee et al., 2018). The authors suggested that increasing the amount of metabolically inactive tissues, such as cuticle, relative to metabolically active tissues, such as muscle, could minimise maintenance costs. Further studies into tissue composition of the rear legs could shed light on the compensatory mechanisms that have evolved in *M. longipes*. An alternative explanation is

behavioural compensation. Under starvation, males bearing larger weapons may reduce their investment in aggressive interactions, thereby limiting additional energetic expenditure. Although floaters remained covered with fertilised eggs in the intense competition treatment, indicating that mating still occurred, it remains unknown whether matings were primarily achieved by dominant males or by smaller males adopting sneaking behaviours (Toubiana & Khila, 2019).

The absence of detectable maintenance costs during adulthood does not necessarily imply that exaggerated rear legs are cost-free. Instead, the main costs may occur during development, particularly during the fifth nymphal instar, when rear leg growth accelerates (Toubiana & Khila, 2019). Producing such disproportionately large structures may increase developmental duration or cause issues during moulting, resulting in malformed limbs. If males with long rear legs take a longer time to develop, smaller ones might have the opportunity to reproduce, potentially maintaining variation. Developmental investment in exaggerated weapons may also come at the expense of other fitness-related traits, such as spermatogenesis or flight muscle development (Cavender et al., 2021; Simmons & Emlen, 2006). Because wing polymorphism occurs in *M. longipes*, it would be particularly interesting to determine whether investment in flight capability trades-off with weapon exaggeration. Moreover, *M. longipes* live in ephemeral puddles that vary greatly across time and space. Thus, population density, resource availability or the distribution of egg-laying sites could alter the intensity of competition among males and therefore the selective advantage of exaggerated weapons (Toubiana & Khila, 2019). Consequently, even though the reproductive advantage of longer rear legs is considerable, fluctuating ecological conditions may favour the persistence of alternative phenotypes across populations or generations, thereby also contributing to the maintenance of phenotypic variation. Understanding why extensive phenotypic variation persists despite strong directional sexual selection remains one of the central questions in the evolution of sexually selected traits.

Addressing this will require investigating population dynamics, costs acting at other life states or under different ecological contexts, as well as the genetic and developmental mechanisms underlying weapon exaggeration.

Supplementary figures

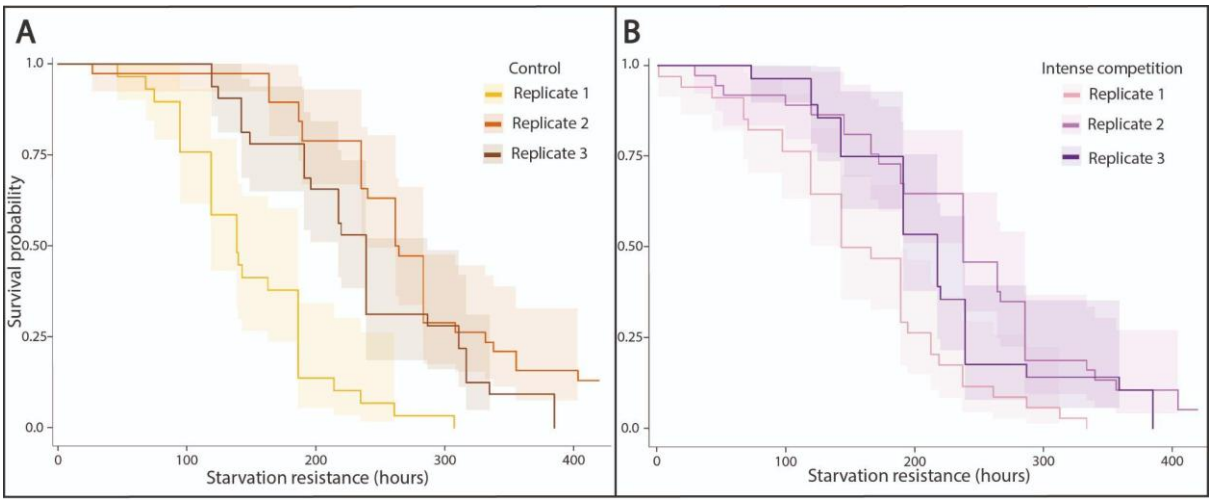

Figure S1. Predicted survival curves for males from the control and the intense competition conditions. Curves were obtained from a Cox proportional hazards model. Shaded areas represent 95% confidence intervals. (A) Predicted curves for the different replicates from the control condition. (B) Predicted curves for the different replicates from the intense competition condition.

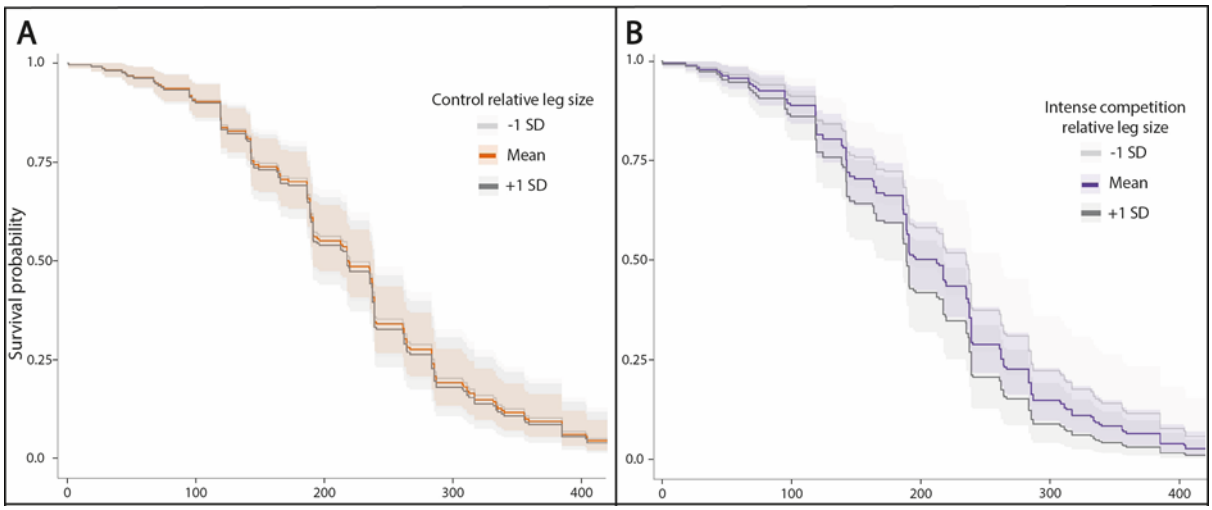

Figure S2. Predicted survival curves for males from the control and the intense competition conditions. Curves were obtained from a Cox proportional hazards model. Relative leg length was standardised prior to analysis (mean = 0, SD = 1), and curves correspond to individuals with relative leg length values one standard deviation below the population mean (−1 SD), equal to the population mean (Mean), and one standard deviation above the population mean (+1 SD). Shaded areas represent 95% confidence intervals. (A) Predicted curves for males from the control condition. (B) Predicted curves for males under intense competition.

Chapter 2

Sexual conflict, directional sexual selection and phenotypic plasticity jointly drive the evolution of extreme phenotypic variation

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Abstract

The persistence of broad variation despite strong and directional sexual selection has been a central question in evolutionary biology. Several mechanisms have been proposed to explain how variation in sexually selected traits can be maintained, by acting on different components of the evolutionary process. For instance, the genic capture model attempts to resolve this paradox by proposing that genetic variation is maintained through capture of genetic variation that modulates an individual's condition. However, empirical evidence supporting these models remains limited. Here, we show that the interaction between sexual conflict, phenotypic plasticity and directional sexual selection generates extreme phenotypic variation in a male weapon. In the water strider *Microvelia longipes*, males exhibit striking variation in the length of their rear legs used as weapons in male–male competition. Using inbred lines and reaction norm experiments, we show that phenotypic plasticity alone fails to explain the broad variation in leg length observed in this species. Experimental evolution applying strong directional sexual selection increased mean leg length but also amplified phenotypic variation beyond the initial distribution. Strong sexual selection also favours nucleotide diversity on the sex chromosome. We further demonstrate that selection favouring long-legged males generates substantial fecundity costs in females, revealing sexually antagonistic selection as an evolutionary force preventing fixation of the exaggerated phenotype. We propose that phenotypic plasticity, acting as a mechanism resolving sexual conflict, amplifies this variation, producing the extreme trait distribution observed in natural populations. Together, these results indicate that the joint action of sexually antagonistic selection, condition dependence and directional sexual selection can inflate phenotypic variation beyond expectations from genetic variance alone, providing an empirical explanation for the evolution of extreme variation in exaggerated secondary sexual traits.

Résumé

Le maintien d'une forte variation phénotypique malgré une sélection sexuelle intense et directionnelle constitue l'une des grandes questions de la biologie évolutive. Plusieurs mécanismes ont été proposés pour expliquer le maintien de la variation des caractères soumis à la sélection sexuelle, chacun agissant sur différentes composantes du processus évolutif. Par exemple, le modèle de la capture de condition (genic capture) propose que la variation génétique soit maintenue parce que les caractères sexuels dépendent de l'état physiologique des individus, lui-même influencé par un grand nombre de loci génétiques. Toutefois, les preuves empiriques permettant de départager ces différents modèles restent encore limitées. Dans cette étude, nous montrons que l'interaction entre le conflit sexuel, la plasticité phénotypique et la sélection sexuelle directionnelle peut générer une variation phénotypique extrême d'une arme sexuelle. Chez la punaise d'eau *Microvelia longipes*, les mâles présentent une variation remarquable de la longueur de leurs pattes postérieures, utilisées comme armes lors des compétitions entre mâles. À l'aide de lignées consanguines et d'expériences de normes de réaction, nous montrons que la plasticité phénotypique, à elle seule, ne permet pas d'expliquer l'ampleur de la variation observée dans la longueur des pattes. Une expérience d'évolution expérimentale, imposant une forte sélection sexuelle directionnelle, a entraîné une augmentation de la longueur moyenne des pattes, mais également une amplification de la variation phénotypique au-delà de la distribution initiale. Cette forte sélection sexuelle s'accompagne également d'un maintien plus important de la diversité nucléotidique sur le chromosome sexuel. Nous montrons par ailleurs que la sélection favorisant les mâles aux longues pattes engendre d'importants coûts de fécondité chez les femelles, révélant l'existence d'une sélection sexuellement antagoniste susceptible d'empêcher la fixation des allèles associés au phénotype exagéré. Nous proposons que la plasticité phénotypique, en contribuant à atténuer les effets du conflit sexuel, amplifie cette variation et participe ainsi à la production de la distribution extrême des tailles de pattes observée dans les populations naturelles. Dans leur ensemble, ces résultats indiquent que l'action conjointe de la sélection sexuellement antagoniste, de la dépendance à la condition et de la sélection sexuelle directionnelle peut accroître la variation phénotypique au-delà de celle attendue à partir de la seule variance génétique, apportant ainsi une explication empirique à l'évolution d'une variation extrême des caractères sexuels secondaires exagérés.

Introduction

Exaggerated secondary sexual traits are widespread and often display extraordinary levels of phenotypic variation within populations (Kotiaho, 2001; Pomiankowski & Møller, 1995). In addition to being highly variable, these traits are typically condition-dependent, such that their expression is strongly influenced by environmental conditions experienced during development (Andersson & Iwasa, 1996). Consequently, the remarkable variation observed in sexually selected traits arises from the combined effects of genetic variation and phenotypic plasticity.

The persistence of such extensive variation nevertheless represents a long-standing question in evolutionary biology. Exaggerated secondary sexual traits are generally subject to strong directional sexual selection; theory predicts that genetic variation should progressively decline as alleles associated with increased trait expression become fixed (Kirkpatrick & Ryan, 1991; Pomiankowski & Møller, 1995). Yet substantial phenotypic variation is maintained within natural populations, suggesting that additional evolutionary processes counteract the erosion of diversity expected under persistent selection.

Several hypotheses have been proposed to explain this apparent paradox. One influential hypothesis is the genic capture model (Rowe & Houle, 1997), which proposes that sexually selected traits reflect an individual's overall condition, which is itself determined by a large number of loci. Since condition is influenced by a large number of loci affecting resource acquisition, metabolism, and allocation, sexually selected traits should indirectly capture genome-wide genetic variation. Consistent with this prediction, experimental evolution in bulb mites showed that selection favouring exaggerated male traits captures genetic variation from across the genome while simultaneously reducing overall genomic diversity (Parrett et al., 2022). Other mechanisms may also contribute to the maintenance of variation. Sexually antagonistic selection, for example, can preserve genetic diversity when alleles increasing male reproductive success reduce female fitness, preventing their fixation within populations (Chippindale et al., 2001; Connallon & Clark, 2014). Likewise, genotype-by-environment interactions and phenotypic plasticity may amplify phenotypic differences by generating different trait expressions across environmental conditions (Kokko & Heubel, 2008). These mechanisms are not mutually exclusive and may act simultaneously, yet their relative contribution to the maintenance of variation in exaggerated sexual traits remains poorly understood.

Despite considerable theoretical progress, empirical studies capable of disentangling the contributions of these mechanisms remain scarce. Disentangling the respective roles of standing genetic variation, phenotypic plasticity, and sexually antagonistic selection is therefore essential for understanding how exaggerated traits maintain high phenotypic variation under directional sexual selection. Such integrative approaches are necessary to better understand how exaggerated sexually selected traits maintain substantial phenotypic variation despite sustained directional selection.

William Rice's 1984 seminal study demonstrated that X-linkage should facilitate the increase in frequency of sexually antagonistic loci; *i.e.* loci with fitness benefits to one sex but that impose fitness costs to the other sex (Rice, 1984). Variation in these genes, if located on the X chromosome, is expected to spread even when their cost to one sex far exceeds their benefit to the other (Rice, 1984). Therefore, a key theoretical prediction is that the sex chromosomes represent a hotspot for the accumulation of sexually antagonistic genetic polymorphism (Rice, 1984). Although a few studies lent support to these predictions, many others failed to uncover these patterns, with some concluding that the X chromosomes are rather cold spots for sexually antagonistic variation (Flintham et al., 2025; Ruzicka & Connallon, 2020).

Here, we investigate the forces underlying the evolution of extreme growth variation in the water strider *Microvelia longipes* (Toubiana & Khila, 2019). Males of *M. longipes* display striking variation in the length of their rear legs, which function as weapons during male–male competition (Toubiana & Khila, 2019). Previous studies have shown that males with elongated legs are more successful in dominating egg-laying sites, securing copulations with gravid females, and siring the majority of offspring (Toubiana & Khila, 2019). While these findings strongly implicate directional sexual selection in favouring the long-legged males, the persistence of small and intermediate males accounting for the striking variation in leg length remains unexplained. We show that phenotypic plasticity alone fails to explain the broad variation observed in male rear leg length. Experimental evolution favouring exaggeration revealed that directional sexual selection not only fails to erode variation, but rather is necessary to maintain variation in male weapon and nucleotide diversity on the sex chromosome. Furthermore, directional sexual selection favouring male weapon also imposes fecundity costs on females, highlighting the role of sexually antagonistic selection in generating variation in this trait.

Materials & Methods

Study organism

Microvelia longipes is a gregarious species that lives in small temporary puddles filled with rainwater in tropical regions of South America (Andersen, 1982; Toubiana & Khila, 2019). Females lay their eggs on debris of twigs and dead leaves floating on the surface of water, which we refer to as egg-laying sites (Toubiana & Khila, 2019). Males guard these egg-laying sites, signal to attract females, and fight using their 3rd pair of legs as weapons to fend off competitors (Toubiana & Khila, 2019). Males with long 3rd pair of legs are favoured by intrasexual competition, which has led to the evolution of exaggerated leg length (Toubiana et al., 2021; Toubiana & Khila, 2019). Moreover, both genetic variation and phenotypic plasticity in response to nutritional treatment during nymphal development contribute to the high phenotypic variation observed in leg length in wild populations (Toubiana & Khila, 2019).

Nutritional treatment of the inbred lines:

The males of the three inbred lines (LH, LL and SH) used in this paper were the descendants of the same inbred lines generated in Toubiana & Khila (2019). For each inbred lines first instar nymphs were collected just after hatching and reared in either poor or rich nutritional condition. For both nutritional conditions, a hundred first instar nymphal instar nymphs were fed with 4 fresh crickets per day. During the second nymphal instar, 20 individuals were fed 1 frozen cricket leg per day in the poor condition, whereas in the rich condition they were fed 2 fresh crickets. In the poor condition, 50 individuals were fed 1 frozen cricket leg per day for the rest of their nymphal development. In the rich condition, 10 individuals were fed 2 fresh crickets per day for the rest of their nymphal development. Once males reached adulthood, they were sacrificed in 70% ethanol. Then 75 males per inbred line and nutritional condition were dissected to isolate the rear leg from the body. Rear legs were removed from the body at the coxa, then they were mounted between a slide and a cover slip in Hoyer's solution and dried at 60 °C for 2 hours. For each individual, the pair of rear legs was measured from the coxa to the tip of the tarsus with the Keyence macroscope VHX 7000. Then, the body was measured using the Zeiss macroscope SteREO Discovery V12 from the pronotum (thorax) to the tip of the 6th abdominal segment; the head and the genital organs were not measured because they are flexible and can bias the measurements. Males from the Brazil and French Guiana populations were dissected and measured in the same manner as individuals from the inbred lines.

Experimental evolution

A wild population composed of 50 females and 41 males collected in 3 locations in French Guiana in April 2023 (GPS coordinates: 4.30275, -52.13956; 4.04089, -52.02277; 3.90165, -51.81666) was allowed to expand for one generation in the laboratory to generate a base population. From this base population, eight replicate experimental evolution populations were established, four subjected to selection favouring dominant males with long legs (Selection lines), and four in which sexual selection was relaxed (Control lines). Each population was founded by 260 recently emerged adults from the base population (130 random females and 130 random males) (Supplementary Fig. S1).

Selection regime for dominant males:

In *M. longipes*, males compete for egg-laying sites and large males almost always win (Toubiana & Khila, 2019). We therefore base our selection regime on this mating system by reducing the number of egg-laying sites to 1 site per 10 males to amplify competition that favours dominant males (Emlen, 2001; Toubiana & Khila, 2019). The size of these sites was also reduced so that only one male at a time can defend it (1 cm x 0.10 cm). In addition to intensified competition, we also sought to increase selection efficiency by limiting the effect of phenotypic plasticity that hides the genotype from selection. We therefore subjected the selection line to a standard diet (frozen crickets) during the nymphal stages (nymphal instars 1 to 5). This treatment, in our experience, is less rich and is meant to enhance the congruence between the genotype and the phenotype. The nymphs from the selection line were therefore fed once a day with frozen crickets, in the same way as species stock are maintained in the laboratory (Table 1).

Control line:

Control replicates were maintained in conditions of low or no competition for egg-laying sites. They were provided with Styrofoam stripes (2 x 8 cm), which are 160 times larger than the egg-laying sites provided to the selection lines. The size of these floaters makes it impossible for males to defend. This ensures that females lay eggs without having to copulate with a dominant male. In addition, the nymphs from control lines were fed *ad libitum*, twice a day with fresh crickets, in order to amplify phenotypic plasticity (Table 1).

For both lines, once the nymphs reached adulthood, they were fed the same standard diet. The populations were maintained at 28-30°C, 50-60% humidity and under an artificial photoperiod of 14 hours of daylight in water tanks (37x54x21 cm). Eggs laid on the egg-laying

sites (Selection line) or floaters (Control line) were allowed to develop to constitute the next generation. The adults were allowed to interact freely until there were enough nymphs to constitute the next generation (~260 individuals for each population), then they were photographed for subsequent phenotyping and sacrificed in absolute ethanol before being frozen at -70 °C (Supplementary Fig. S1).

To disentangle the effects of selection regime from developmental nutrition, individuals from both lines were reared under identical nutritional conditions during G15. For each replicate of each line, nymphs were randomly assigned to a diet (*Ad libitum* diet or standard diet).

From generation 17 on, both lines were maintained at a less intensive level: smaller tanks, smaller population, fed with a standard diet (once a day with frozen crickets), but we maintained competition by providing small egg-laying sites to the selection lines and large Styrofoam floaters to the controls.

	Base population	**G1**	**G5**	**G10**	**G15**	**G16**	**G17**
SLL lines	Rich diet	Standard diet	Standard diet	Standard diet	Rich and Standard diet	Rich and Standard diet	Standard diet
Control lines	Rich diet	Rich diet	Rich diet	Rich diet	Rich and Standard diet	Rich and Standard diet	Standard diet

Table 1: Nutritional diet for both lines throughout the experiment

Phenotyping:

Due to a large number of individuals per generation (~2 000 across all replicates), we chose a group imaging method that is compatible with quick but reliable measurements. This method is dependable because the insects adopt a stereotypical posture naturally on the water surface (Supplementary Fig. S2). At each generation, we subdivided each population into multiple groups of approximately 50 living animals, and pictures were taken using a vertically placed Nikon D7200 camera with an AF-S Micro-NIKKOR 105 mm lens. The length of the body, the tibia and the femur of both sexes were measured for the wild population captured in

2023 and for all replicates from the base population and at G1, G5, G10 and G15 using the software ImageJ (v1. 53t). Body length was measured from the tip of the head to the tip of the genitalia. Tibia length from the joint with the tarsus to the joint with the femur, and femur the joint with the tibia to the joint between the femur and the side of the body (Supplementary Fig. S2).

Fecundity assay

To test if selection favouring dominant males had any impact on female fitness, we tested fecundity of the females in both control and selection lines. Four types of crossings were set up (male SLL line x female SLL line; male Control line x female SLL line; male SLL line x female Control line; male Control line x female Control line), each with thirty biological replicates consisting of a single couple each. For each replicate, one male and one female were allowed to mate in a plastic container (14x5x8 cm) filled with water, and two cork egg-laying sites were made available (each: 1.5 cm x 0.1 cm). Egg-laying sites were changed every two days for 12 days, and the total number of eggs laid and the number of eggs that hatched were counted 7 days after the first hatching (embryonic development takes about 5 days). A period of 12 days was chosen, prior to data analysis, as this was the longest period during which no females had yet died. The average lifespan of an adult female, since emergence, is around 34 days (unpublished data). Individuals were isolated at the 5th instar to standardise the age of adults and capture most of female lifetime fecundity. When a male died during the experiment, it was replaced by a male from the same line. This experiment was performed twice, once in April 2025 at generation 23 for the SLL line and generation 27 for the Control line with 10 replicates per crossing, then in July 2025 at generation 27 for the SLL line and generation 31 for the Control line, with 20 replicates per crossing (Supplementary Fig. S1). Egg-laying sites used for the selection lines were smaller, which increased a bit the time required to produce the next generation, thus causing a delay between the two lines.

Statistical analyses

Statistical analyses were conducted using R software (v.4.2.2, *R Core Team 2022*) and data were visualised using ggplot2 (v.3.3.6). The comparison of variance of body size and leg length between the inbred lines and natural populations was done with the R package cvequality v.0.2.0 (Marwick & Krishnamoorthy, 2019).

Log-transformed data were used for scaling relationship comparison, and we performed a linear mixed-effects model, with the interaction between the generation and the condition as the predictor and the replicates and the person measuring as random effects. We ran a similar model for females.

Comparisons for trait distributions were also performed on log-transformed data. We tested whether the length of the tibia of the rear leg and the dispersion (proxy of the total leg length) (Toubiana and Khila, 2019) changed across generations due to the experimental condition using a generalised linear mixed model with tibia length as the dependent variable, the interaction between the generation and the condition as the predictor and the replicates and the person measuring as random effects ('glmmTMB' package, v.1.1.11, Brooks et al., 2017). Similar models were run for the length of the tibia of the rear leg in females and body length in both sexes. At generation 15, we reared individuals from both lines under identical nutritional conditions to disentangle the effects of selection regime from nutritional treatment. In order to test for the effects of these parameters on tibia and body length in individuals at generation 15, we used a linear mixed-effects model with the length of the body or tibia as the dependent variable, the interaction between the diet and selection regime as the predictor and the replicates and the person measuring as random effects.

The effect of the crosses on the total number of eggs laid by females was tested using a negative binomial generalised linear mixed model with a log link function ("glmmTMB" package, v.1.1.11, Brooks et al., 2017). The probability of hatching was also compared between crosses using a beta-binomial mixed model ("glmmTMB" package, v.1.1.11, (Brooks et al., 2017). The month of the experiment was included as a random effect for both models. Model adequacy was assessed using the DHARMa package (v.0.4.7, Hartig, 2024) residual diagnostics, which indicated no remaining overdispersion or deviation from model assumptions.

DNA extraction & Sequencing

For genomic analyses, we sampled material from the wild population captured in 2023, from the base population, and from each line at G1, G5, G10 and G15. From each population, two samples were collected, consisting of 50 individuals (1 × 50 females and 1 × 50 males). The 50 individuals within each sample were grinded in liquid nitrogen, and DNA was extracted using the extraction kit Qiagen 20/G according to the manufacturer's instructions. Library

preparation was performed using the TruSeq PCR-free library kit for Illumina, and whole-genome resequencing was carried out by the SciLife SNP&SEQ Technology Platform (Uppsala University, Sweden) using the Illumina NovaSeq X system with a 25B flow cell to produce 2 × 150 bp paired-end reads. The target coverage for the samples from the wild population and from the base population was 100x, and for G5, G10 and G15 was 50x for a genome of 670 Mb (Toubiana et al., 2021).

Mapping pipeline and variant calling

All sequenced libraries were of high quality, with average raw PHRED-scaled base qualities exceeding XX in the 82 samples. Thus, raw FASTQ reads from all samples were trimmed and filtered using *fastp* to retain very high-quality bases (minimum base PHRED quality = 25; minimum sequence length = 120 bp). We discarded unpaired reads, and we conserved read pairs where both reads fulfilled all quality criteria. Then, we mapped the retained reads against the assembled genome of *Microvelia longipes* using *bwa mem* (v.0.7.18). For all samples, the files were converted into BAM files and sorted using *samtools* (v.1.21). After that, we removed optical duplicates with *Picard*. Then, we generated a target list of indel positions and re-aligned the reads around them using *GATK* (v.3.8). We used the *Mpileup2Sync* Python script from DEST v2 (Kapun et al., 2021) to merge the BAM files from all the samples and convert them into a sync file. We performed heuristic SNP calling using a version of *PoolSNP* (Kapun et al., 2020) adapted for sync files (Kapun et al., 2021), with the following parameters: minimum coverage from all samples = 10x, maximum coverage from all samples = the 95th coverage percentile for a given chromosome and sample, minimum read count for a given allele = 20 x, minimum read frequency of a given allele = 0.02, finally, ≥ 10% of all samples that did not meet the minimum and maximum coverage threshold were discarded. We also excluded positions that overlapped with indels or were located within 5 bp of them, as well as positions located within known transposable elements (TEs) based on a *M. longipes* TE library.

Population Genetic analyses

To characterise the extent and distribution of genetic variation in each sample, we estimated the nucleotide diversity (Tajima's Pi, π) focusing on SNPs located on the autosomal chromosomes. First, all regions were analysed, then coding regions. Window-wise average of 50 kb length

with 10 kb step-size with *Grenedalf* (Czech et al., 2022), which employs statistical corrections for population genetics estimators as described in Kofler, et al. (2011). We ran a linear mixed-effects model with the Tajima's Pi value as the dependent variable, the interaction between the generation, the selection regime and the sex as the predictor and the replicates and the contigs as random effects. Then, we repeated the same analyses for sexual chromosomes.

Results

Alone, phenotypic plasticity fails to explain the broad variation in male leg length.

The extent to which condition dependence expands standing genetic variation into extreme leg length variation in the males of *Microvelia longipes* is unclear. We therefore conducted a series of experiments aimed at isolating the effect of nutrition-driven plasticity from that of genetic variation on male leg length variation. Distinct natural populations of *Microvelia longipes* from French Guiana (FG) and Brazil showed high and consistent male leg length variation with a coefficient of variance of 25 % and 29 %, respectively, and a mean male leg length of 6439 µm and 6542 µm (Fig. 1). The coefficient of variance of body size in the FG and Brazil populations was 7.8 % and 8.8 %, respectively.

Next, we examined leg length variation in three inbred lines derived from the French Guiana population and generated through 20 generations of brother-sister crosses of randomly chosen males at each generation (Toubiana and Khila, 2019). The coefficients of variance of male leg length and body size of these lines dropped dramatically, in both poor and rich diets, and in some cases by over half in comparison with the initial natural population (Fig. 1).

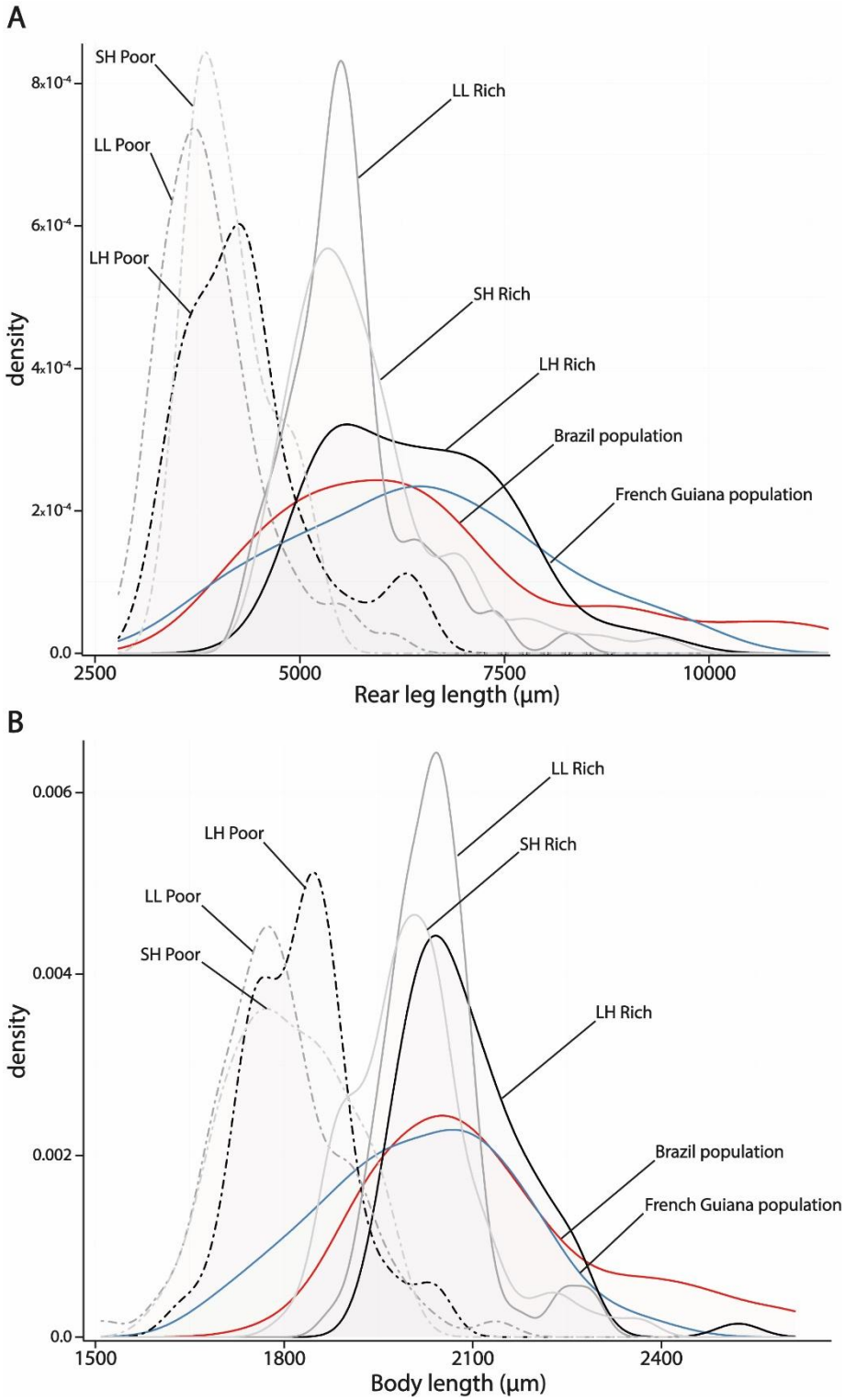

Figure 1. Effect of plasticity and genetic variation on the variation in rear leg length (**A**) and body length (**B**) in the males of *Microvelia longipes*. Red represents a wild population collected in Brazil in 2015 and blue a wild population collected in French Guiana in 2013. Light grey, dark grey and black represent three inbred lines derived from the French Guiana population. Continuous lines represent the rich diet, and dashed lines represent the poor diet treatments.

Selection favouring dominant males is necessary to maintain variation in male weapons

Next, we sought to identify the mechanisms responsible for the persistence of both small and large males in the population. Long-legged males dominate egg-laying sites and sire most of the eggs laid on these sites (Toubiana & Khila, 2019). If the accumulation of alleles associated with long legs is driven by dominant males, we should expect an increase in mean male leg length and a decrease in the variation of this trait in populations subjected to directional sexual selection in favour of exaggeration. Conversely, we should expect mean leg length to decrease and the variance to remain high in populations under relaxed selection. To test these hypotheses, we conducted a large-scale experimental evolution study designed to reduce the effects of phenotypic plasticity while imposing strong directional selection favouring long-legged males (see methods for detail). As controls, we created lines where male-male competition is relaxed and high rear leg length variation was favoured (see methods).

At the start of the experiment, males from both control and selection lines showed no difference in rear tibia or body length (rear tibia length $p = 0.732$, body length $p = 0.931$) (Fig. 2A and B). This also indicates that there was no bias when lines and replicates were created. At G1, males from the control line had rear legs 8.6% longer ($p < 0.0001$), while males from the selection line had overall slightly smaller legs ($p < 0.0001$) when compared to the base population (Fig. 2A). This is consistent with the differences in nutritional treatment applied to the control and selection lines (see methods for detail). Between generations 5 and 10, mean male rear leg length in the selection lines caught up and exceeded that of control lines, even though control lines were raised on a richer diet. This difference between the experimental lines persisted at generation 15 (Fig. 2A). Although the mean length of male rear legs in the selection lines increased over generations, it did not exceed that of the base population at this generation ($p = 0.0514$). Thus, this divergence is mainly due to the decrease in the mean in the control line, in which males from generation 15 had rear legs that were 11.8% shorter than the base population ($p < 0.0001$) (Fig. 2A). Surprisingly, the effect of experimental evolution on male rear leg length variation was contrary to our expectations. At generation 15, variation in this male weapon has increased by 16% in the selection line ($p = 0.0164$) and has decreased by 47% in the control line ($p < 0.0001$) compared to the base population (Fig. 2A). In both lines, the mean of male body length showed the same pattern as rear leg length, consistent with previous work establishing that these two traits covary. However, the effect size on the body is much smaller than for the length of the rear legs. Specifically, the difference in body length between

males from the selection and the control lines was up to 4% ($p < 0.0001$) at generation 15 (Fig. 2B). This indicates that body length responded to selection favouring dominant males with a lower rate compared to rear leg length. Conversely to rear leg length, the variation of the body length did not vary significantly between generations in either line (selection line $p = 0.3962$, control line $p = 0.5784$), although there was a 19% difference in body length ($p < 0.0001$) between the selection and the control lines at generation 15 (Fig. 2B).

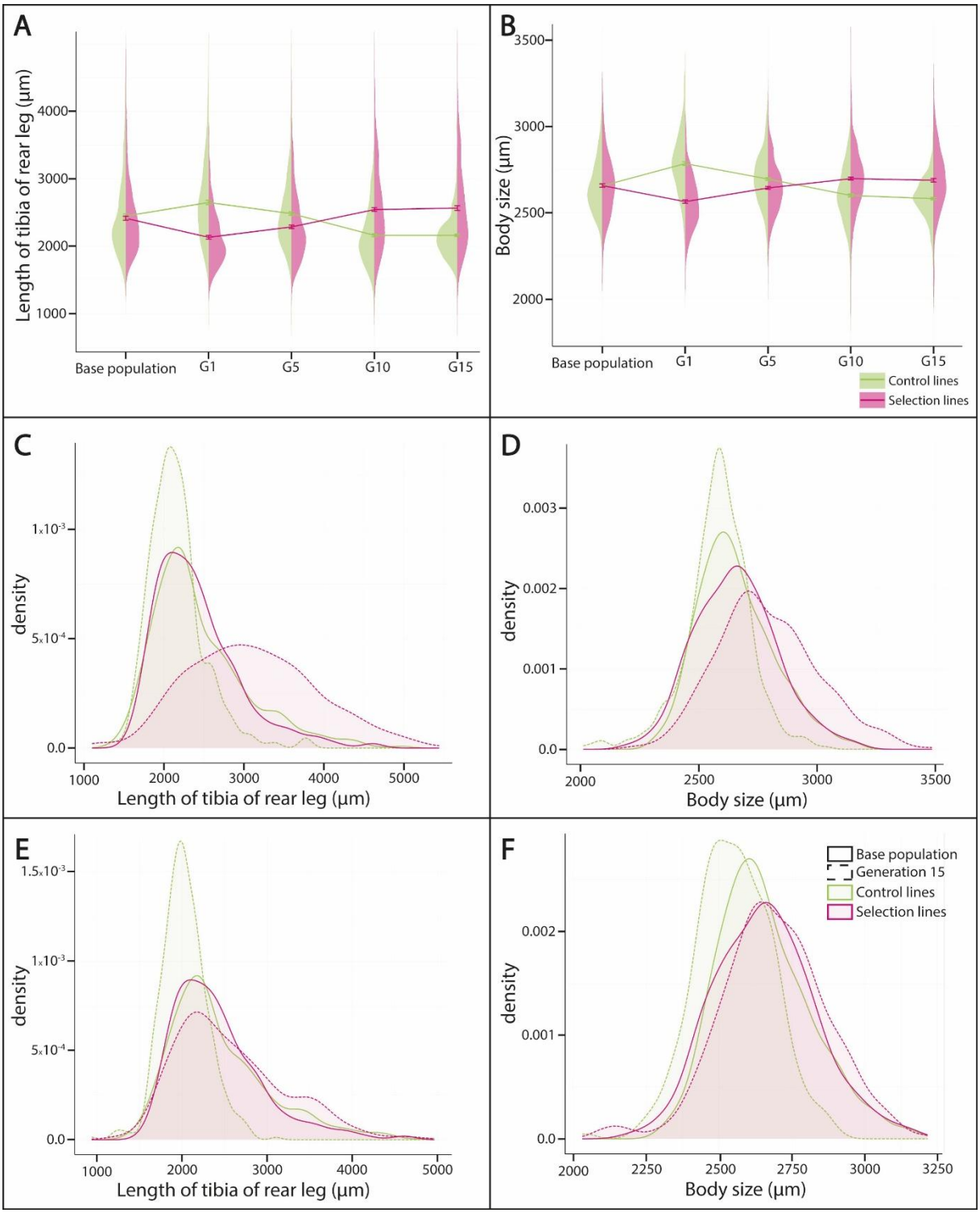

Figure 2. (A) evolution of the length of the rear leg in males, the mean ± standard error is represented (B) evolution of the body length in males, the mean ± standard error is represented (C) and (D) distributions of the length of the rear tibia and body at G15 in the *ad libitum* diet, (E) and (F) distributions of the length of the leg and body at G15 in the standard diet. Green represents the control lines, and purple the selection lines. Plain lines represent the base populations and the dashed lines generation 15.

Females from the selection lines also responded to the standard diet since they had legs 6.8% ($p < 0.0001$) and bodies 2.5% smaller ($p = 0.0001$) than females from the base population. Female rear leg length and body length responded differently to the experimental conditions than males (Supplementary Fig. S3A and B). In both lines, mean rear leg length decreased till generation 10 ($p = 0.1809$), and then increased from generation 10 to 15 ($p = 0.1440$) to reach a similar length compared to the base population (Supplementary Fig. S3A). In the selection line, the variation in females' leg length was not impacted by the experimental condition over generations (all p-values > 0.300). Contrastingly, there was an increase in variation at generation 5 and generation 10 up to 33% ($p < 0.0001$), then it reduced between generation 10 and generation 15 to reach the same value of dispersion as the base population ($p = 1.00$). Between the two lines, the evolution of mean body length follows the same pattern as the rear legs; they diverged until generation 10 ($p = 0.4424$) and stayed similar at generation 15 ($p = 0.1431$). However, both lines were smaller than the base population. Interestingly, variation in body length decreased by 40% ($p < 0.0001$) between the base population and generation 15 in the control line, whereas in the selection line body length variation was similar to the base population (Supplementary Fig. S3B).

Selection favouring dominant males increases the allometric coefficient of weapon

To disentangle the effects of sexual selection from nutritional environment, individuals from both lines at generation 15 were reared under identical dietary conditions (standard and rich) during nymphal development. For both lines, males reared on a rich diet had longer legs and bodies than males fed on the standard, less rich diet. Interestingly, the effect size of the diet was greater in the selection line, in which males on the rich diet had rear legs 20% longer ($p < 0.0001$), whereas in the control line they only differed by 5.5% ($p < 0.0001$) (Fig. 2C). This further supports the conclusion that phenotypic plasticity will expand standing genetic variation into higher phenotypic variation.

When we compared lines fed on a rich diet, males from the selection line had legs 27.9% longer ($p < 0.0001$) and bodies 5.8% longer than males from the base population (Fig. 2C and D). Conversely, males from control lines have rear legs 11.5% shorter and bodies 2.8% shorter than the base population (Fig. 2D and F). Males from selection lines had rear legs and bodies 41.2% and 8.9% ($p < 0.0001$) longer than control lines at generation 15. Moreover, the rear legs

of males from the selection line were 43% ($p < 0.0001$) more variable than males from the control line at generation 15 (Supplementary Table 1).

The allometric coefficient of covariation between rear legs and body also responded to selection favouring dominant males. By generation 15, the allometric coefficient of the control lines had decreased relative to the base population (Fig. 3A and B; 2.0 for the rich diet, $p < 0.0001$). In contrast, males from the selection lines maintained a high allometric coefficient (Fig. 3A and B). At generation 15, the allometric coefficient was also significantly higher in the selection line compared to the control line ($p < 0.0001$). The effect of replicates on the results was limited, as there was no significant difference between them (Supplementary Fig. S5A). These data indicate that the two treatments diverged in opposite directions from the ancestral population and that selection favouring dominant males is necessary to maintain a high mean and variance of weapon size (Fig. 2C, 3A and B).

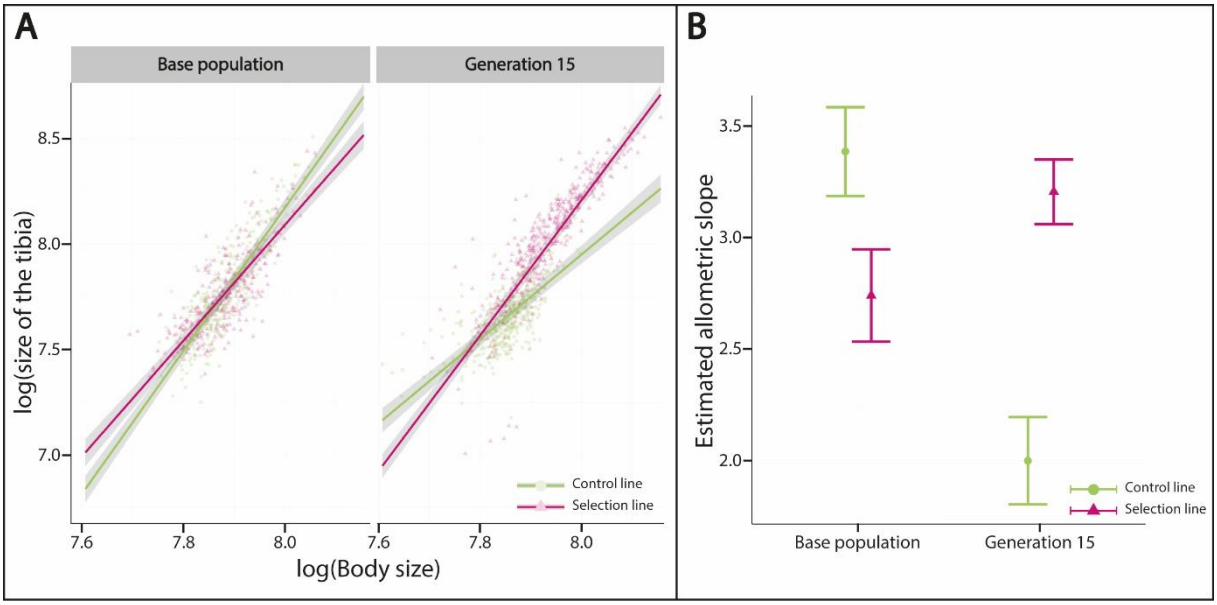

Figure 3. (A) Scaling relationships of log-transformed data between rear legs and body sizes estimated in males fed on a rich diet from the two lines from the base population and generation 15. (B) Allometric slopes of log-transformed data between rear leg and body lengths estimated in males fed on a rich diet from the two lines from the base population and generation 15, bars represent 95% confidence intervals. Control lines (green and circle), selection lines (purple and triangle).

Under the same nutritional treatments at generation 15, there was no difference in the length of female rear legs or body between the lines (all p-values > 0.08, Supplementary Fig. S3 C, D, E and F). However, within each experimental line, females fed on a rich diet have slightly longer rear legs (1.1% longer in rich compared to standard in the control lines ($p =$

0.0296) and in the selection line 1.4% longer (p = 0.0178)). In females, we found no difference in allometric coefficient between the lines and over generations (Supplementary Fig. S4A and B).

Altogether, these results indicate that, contrary to theoretical predictions for exaggerated secondary sexual traits, directional sexual selection not only fails to reduce variation in male weapon size, but is rather necessary to maintain it in *Microvelia longipes*.

Sexual selection favouring dominant males maintains nucleotide diversity on the sexual chromosome in males

The nucleotide diversity (π) was higher for individuals of the base population relative to the wild population for both sexes (Supplementary Fig. 6). Across the experimental evolution, nucleotide diversity (π) generally declined compared to the base population, although the magnitude of this decline depended on sex, genomic compartment and selection regime. In autosomes, females from both control and selection lines exhibited a significant reduction in nucleotide diversity throughout the experiment compared with the base population (all $p < 0.0001$). Males also showed an overall reduction in autosomal diversity relative to the base population, particularly at generation 15 in both control (estimate = -9.55×10^{-4}, $p < 0.0001$) and selection lines (estimate = -8.18×10^{-4}, $P < 0.0001$), although temporal trajectories differed slightly between treatments (Fig. 4A). However, the nucleotide diversity was similar for both conditions at generation 15. Diversity remained consistently higher in SLL females than in Control females at the end of the experiment (G15; estimate = 4.29×10^{-4}, $p < 0.0001$, supplementary Fig. S7A). Similar patterns were observed when considering only coding sequences (CDS), albeit with substantially lower absolute diversity values (Fig.4B and supplementary Fig. S7B)

In contrast, the sex chromosome displayed marked sex-specific patterns. In males, nucleotide diversity decreased markedly throughout the experiment in both selection regimes (Fig. 4 C and D). Control males showed significantly lower diversity than the base population at every sampled generation (all $p < 0.001$), with the strongest reduction observed at generation 15 (estimate = -6.29×10^{-4}). At the end of the experiment, selection males retained significantly higher diversity than control males (estimate = -4.34×10^{-4} for Control − SLL, $p < 0.0001$, Fig. 4C). Restricting the analysis to coding regions of the sex chromosome produced the same

qualitative pattern, with control males exhibiting a stronger reduction in diversity than selection males at generation 15 (estimate = -8.89×10^{-6}, $p = 0.0024$, Fig. 4D).

Females exhibited a markedly different pattern on the sex chromosome. While control females experienced transient reductions in diversity at generation 1 and generation 10 relative to the base population (estimates = -3.37×10^{-4} and -3.09×10^{-4}, respectively; both $p < 0.001$), nucleotide diversity at generation 15 did not differ significantly from the founder population (estimate = -6.49×10^{-5}, $p = 0.27$). In selection lines, females' nucleotide diversity remained remarkably stable across generations, with no significant differences between the base population and any subsequent generation (all $p > 0.65$, supplementary Fig. S7C). The same pattern was observed when analyses were restricted to coding sequences of the sex chromosome, where no temporal change was detected in selection females, whereas control females showed only modest and transient reductions (Supplementary Fig. S7D). Overall, these results indicate that whereas nucleotide diversity declined across most of the genome during experimental evolution, diversity on the sex chromosome was largely maintained in females, while males experienced a pronounced loss of diversity.

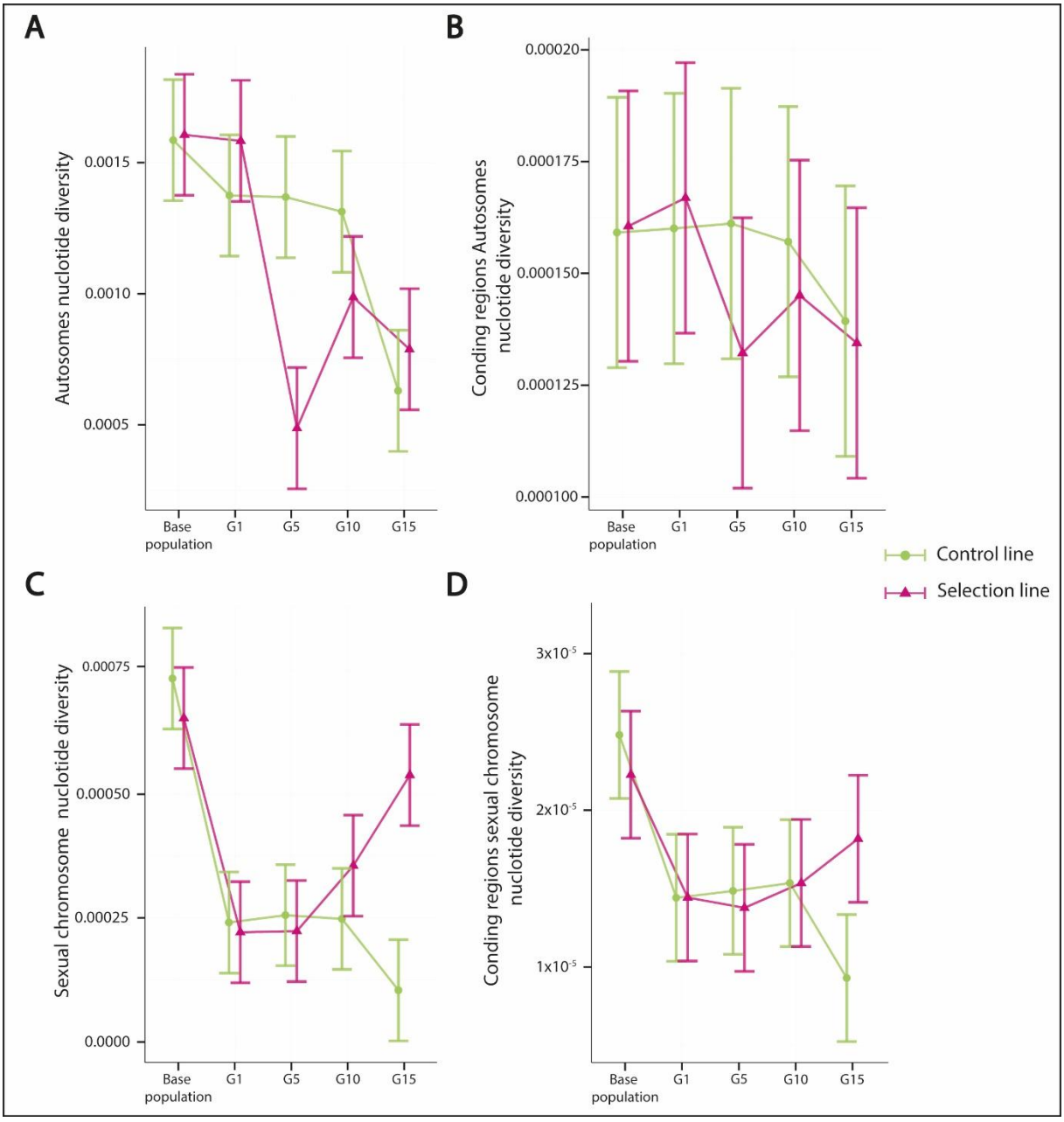

Figure 4. Nucleotide diversity (π) estimated in males from the base population, generation 1, generation 5, generation 10, and generation 15 for both lines. Bars represent 95% confidence intervals. Control lines (green and circle), selection lines (purple and triangle). (A) Nucleotide diversity of autosomes. (B) Nucleotide diversity of autosomes' coding regions. (C) Nucleotide diversity of the sex chromosome. (D) Nucleotide diversity of the sex chromosome's coding regions.

Selection favouring male weapons imposes fecundity costs on females

Sexually antagonistic selection is a powerful evolutionary force that can act in opposite directions in males and females when traits are genetically correlated between the sexes. We therefore hypothesised that the maintenance of high variation in male weapons, in the face of directional sexual selection, could be the consequence of costs to females. To test this hypothesis, we compared female fecundity, a primary fitness component, between selection (long-legged males) and control lines (short-legged males). Females from selection lines laid 23% fewer eggs than females from control lines, regardless of the origin of the males that they mated with (males from selection or control lines). The month of the experiment accounted for a moderate proportion of variance in egg number (ICC = 0.39), indicating that while it contributes to variability, most variation occurs among replicates within a month.

Probability of egg hatching did not differ between treatments, indicating that the fecundity costs experienced by females from selection lines resulted from reduced egg production rather than reduced egg viability. In addition, the month of the experiment explained only about 22% of the total variance in hatching success (ICC = 0.22), with the majority of variability occurring among replicates within a month. These results demonstrate that sexual selection favouring dominant males generates fecundity costs in females. Therefore, sexually antagonistic selection acts in opposition to directional sexual selection, maintaining both large and small males in the population.

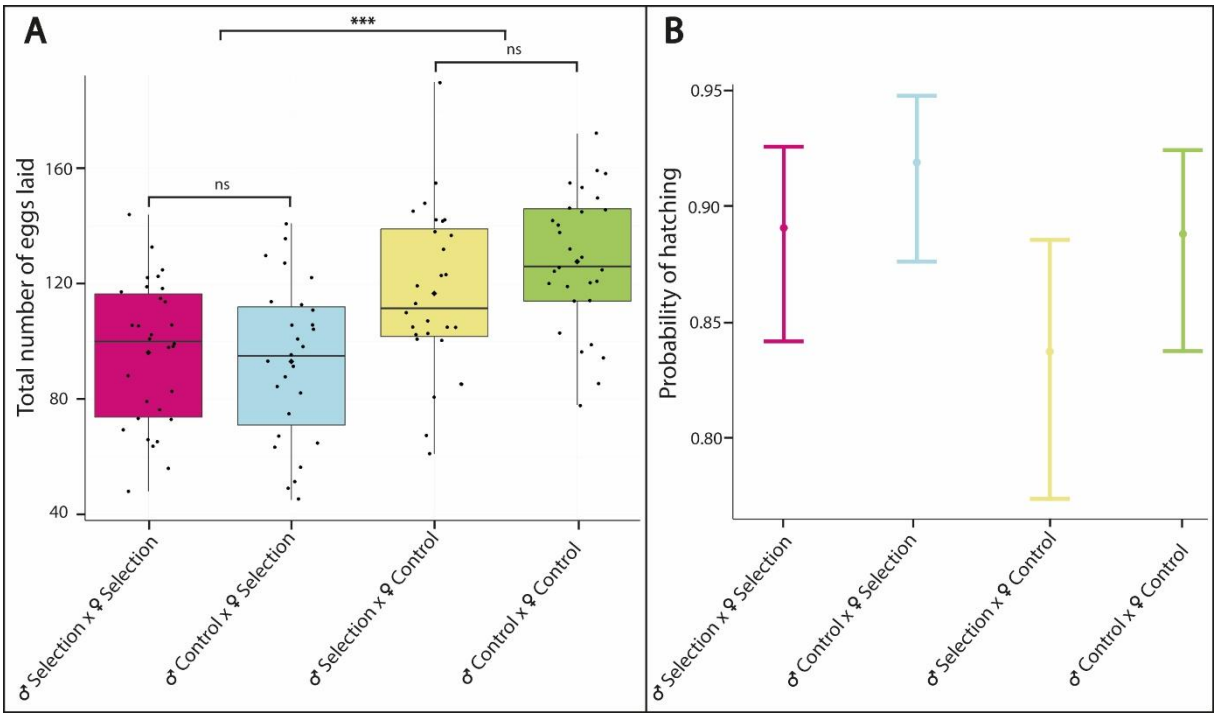

Figure 5. (A) Raw number of eggs laid for the four crossings. (B) Probability of hatching for the four crossings, bars represent 95% confidence intervals for model-estimated probability.

Boxplots display the raw number of eggs per replicate, while reported means are based on model-estimated means. On average, the number of eggs laid over 12 days in the male Selection x female Selection crossing was 89.8 eggs (95% CI: 71.1 – 113), and in the male Control x female Selection crossing, it was 88.3 eggs (95% CI: 69.9 – 112) (Fig. 5A). These two crossings laid a similar number of eggs (ratio = 1.017, 95% CI: p-value = 0.99) (Figure xx, A), however, they laid in average 23% less eggs than females from the Control line (p-value < 0.001) (Fig. 5A). The male Selection x female Control and the Male Control x female Control crossings also laid a similar number of eggs (ratio = 1.086, p-value = 0.41). Furthermore, in all crossings, the hatching success was high (between 0.84 and 0.92), and there was no significant difference between them (p-value > 0.05 between all crossings) (Fig. 5B). As a result of lower egg production and similar hatching probability, females from the male Selection x female Selection and male Control x female Selection crossings had fewer offspring than females from the Control line. Thus, selection favouring dominant males with long legs has a negative impact on female fecundity (no impact from the origin of the male, only that of the female).

Discussion

We have shown that phenotypic plasticity can expand the variation in male weapon dramatically, but only in the presence of high genetic variation. We have also shown that selection favouring dominant males is necessary to maintain variation in male weapon at the expense of female fecundity. These findings provide a compelling set of advances to our understanding of how variation in exaggerated secondary sexual traits is generated and maintained in natural populations.

Role of phenotypic plasticity and genetic variation in trait exaggeration

Our reaction norm experiments reveal that phenotypic plasticity alone is insufficient to explain the extreme variation observed in male rear leg length in *Microvelia longipes*. While environmental factors, such as diet, contribute to expanding phenotypic variation, the dramatic reduction in variation observed in inbred lines, despite controlled nutritional conditions, highlights the critical role of standing genetic variation in generating high phenotypic variation in this male weapon. These data align with the genic capture model (Rowe and Houle 1996), which posits that condition-dependent traits capture genetic variance underlying condition, thereby maintaining high phenotypic diversity. However, our results extend this framework by demonstrating that directional sexual selection is not only compatible with the persistence of variation but is necessary for its maintenance in this system. This challenges the conventional view that strong directional selection should erode variation (Barton & Keightley, 2002; Fisher, 1930), instead suggesting that selection, in particular for complex traits, may interact with genetic architecture to sustain variation in the trait.

The experimental evolution lines provided a unique opportunity to disentangle the effects of selection and plasticity. In the context where directional sexual selection is absent or reduced (control lines), variation in male leg length collapsed regardless of nutritional treatment. Conversely, lines subjected to directional selection for exaggerated leg length not only maintained but increased variation in this trait compared to the starting population and to the control lines. This result suggests that directional sexual selection may be part of a mechanism that actively contributes to the accumulation of genetic polymorphism in condition-dependent traits, possibly by favouring genetic combinations that confer advantages to males through dominance of egg-laying sites and access to females. The increased allometric coefficient in selection lines further supports this conclusion, indicating that selection amplifies

the scaling relationship between weapon size and body size, reinforcing the exaggeration of the trait.

Phenotypic plasticity as an amplifier of genetic variation in a tug-of-war framework

A central insight from our study is that phenotypic plasticity does not merely respond to environmental cues but actively inflates the genetic variation underlying exaggerated secondary sexual traits, particularly when these traits are subject to opposing selective pressures. In *Microvelia longipes*, male rear leg length is simultaneously pulled in two directions: directional sexual selection favours longer legs for male dominance, while sexually antagonistic selection imposes fecundity costs on females, creating a genetic tug-of-war. Phenotypic plasticity serves as a catalyst in this dynamic, translating the resulting genetic variation into broad variation in male weaponry.

Our experimental results demonstrate that plasticity expands the range of phenotypic expression for a given genotype, allowing genetically diverse males to achieve even greater variation in leg length under favourable conditions (e.g., rich diet). For instance, males in the selection lines reared on a rich diet exhibited a 20% increase in leg length compared to those on a standard diet, whereas the effect was only 5.5% in control lines. This suggests that plasticity magnifies the phenotypic consequences of genetic differences, particularly when selection is strong. In this way, plasticity does not just mask or reveal genetic variation—it amplifies it, providing a mechanism for the persistence of extreme traits despite opposing selective forces.

Moreover, the interaction between plasticity and sexually antagonistic selection may further enhance genetic variation. Females in selection lines, which carry the genetic load of alleles favouring long legs in males, experience reduced fecundity. This cost creates a balancing selection pressure that prevents the fixation of extreme alleles, as males with alleles for longer legs (higher dominance) may produce females with lower fecundity and thus lower fitness. Phenotypic plasticity then allows males with intermediate or shorter legs to adjust their phenotype upward in response to environmental conditions (e.g., diet), thereby achieving some competitive advantage without transferring alleles of lower fecundity to their daughters. This plastic compensation ensures that genetic variation is not only preserved but also expressed across a broader phenotypic spectrum.

Finally, the allometric scaling of leg length relative to body size in selection lines, where the slope of the relationship increased under rich diet, further illustrates how plasticity can expand the phenotypic landscape. By allowing genotypes to express a wider range of trait values under different conditions, plasticity effectively inflates the variance generated through the opposing effects of directional sexual selection (favouring males with long legs) and sexually antagonistic selection (favouring more fecund females). This may explain why, despite strong directional selection for longer legs, variation in *M. longipes* remains so large, as plasticity converts this genetic variation into large variation in male weapon, thus providing material for both selection and conflict to operate.

Sexual selection and the maintenance of variation: a paradox resolved?

The persistence of variation in exaggerated secondary sexual traits has long been a paradox in evolutionary biology. Our study provides empirical support for the idea that directional sexual selection can maintain, rather than deplete, genetic variation in such traits. This finding contrasts with theoretical predictions that strong directional selection should reduce variation (Barton & Keightley, 2002; Fisher, 1999; Schlötterer, 2023). However, our results align with emerging evidence that condition-dependent traits may capture and amplify underlying genetic variation (Parrett et al., 2022), particularly when selection targets multiple loci contributing to condition.

The divergence between the selection lines and the control lines in both the mean and variance of leg length suggests that selection favours not only the exaggeration of the trait but also the diversification of male strategies. In *M. longipes*, long-legged males dominate reproductive sites, but the persistence of smaller males, despite their lower competitive success, implies that alternative strategies (e.g., sneaking or satellite behaviours) may be viable. This has been confirmed through observation in *M. longipes,* where small males adopt a sneaking strategy and manage to fertilise a significant number of eggs (Toubiana & Khila, 2019). This observation could also constitute an additional mechanism that contributes to the maintenance of high variation in male weaponry in this species.

Sexually antagonistic selection as a hidden cost of male dominance

A key insight from our study is the fecundity cost imposed on females in lines selected for exaggerated male leg length. Females from selection lines laid 23% fewer eggs than those from control lines, regardless of the male's origin. This demonstrates that sexual selection on

males can have detrimental effects on female fitness, a hallmark of sexually antagonistic selection. Such conflicts arise when traits that enhance male reproductive success incur costs for females, often due to shared genetic architecture between the sexes (Rice, 1984). How this correlation operates at the genomic level remains to be tested. Developmental pathways involved in growth and patterning are known to be highly pleiotropic and often expressed across a number of tissues and developmental stages (Jee et al., 2026; Mackay & Anholt, 2024; Mauro & Ghalambor, 2020). Pleiotropy, the phenomenon where a single genetic variant influences multiple traits, is now recognised as a pervasive feature of genetic architecture (Paaby & Rockman, 2026; Mackay & Anholt, 2024). In *Microvelia longipes*, pleiotropy may underlie the maintenance of variation in male leg length by linking this trait to other fitness-related pathways, such as those governing body size or fecundity. This is likely because, in this species, the gene BMP11 has been shown to regulate leg length and body size in males as well as body size in females (Toubiana et al., 2021). Furthermore, a number of studies have shown that pleiotropic genes are often enriched in fundamental cellular pathways (e.g., cell proliferation, DNA repair, and signalling) (Shikov et al., 2020), which could explain how selection on male weapons imposes costs on female fecundity through shared genetic pathways.

In this context, our results support the hypothesis that sexually antagonistic selection acts as a balancing force, counteracting the directional selection for trait exaggeration in males. By reducing female fecundity, selection on male weapons may limit the spread of alleles favouring extreme leg length, thereby maintaining genetic variation in the population. This mechanism complements the genic capture model, as it provides a selective counterbalance that prevents the fixation of extreme traits. The lack of difference in egg hatching success between treatments further suggests that the cost is primarily mediated through reduced egg production, rather than viability, aligning with previous studies on sexually antagonistic fitness effects (Chippindale et al., 2001).

Role of sex chromosomes and future directions

While our study did not directly test the role of X-linkage in sexually antagonistic variation, our findings contribute to the broader debate on the genomic location of such conflicts. The theoretical prediction that sex chromosomes are hotspots for sexually antagonistic polymorphism (Rice, 1984) has received mixed empirical support (Connallon & Clark, 2014; Flintham et al., 2025).

In *M. longipes*, the persistence of variation in male leg length despite strong selection suggests that multiple genetic mechanisms, including autosomal and sex-linked loci, may interact to maintain trait diversity. Future work could explore the genomic distribution of loci contributing to leg length variation, particularly focusing on regions under sexually antagonistic selection.

Additionally, the allometric scaling of weapons relative to body size responded strongly to selection, with selection lines maintaining higher allometric coefficients. This suggests that selection may target the developmental pathways that regulate trait exaggeration, rather than just the trait itself. Investigating the genetic and hormonal mechanisms underlying these allometric changes could provide further insights into how selection shapes the evolution of exaggerated traits.

The overall decline in nucleotide diversity observed across autosomes is consistent with expectations for experimental evolution studies. Genetic drift and recurrent sampling during population maintenance are expected to reduce neutral genetic diversity over successive generations. Similar losses of diversity have been reported in Evolve-and-Resequence experiments, where founder effects and finite population sizes progressively erode standing genetic variation (Parrett et al., 2022).

An intriguing result of this study is the contrasting evolutionary trajectories of nucleotide diversity on the sex chromosome between control and selection males, as stronger directional sexual selection is generally predicted to erode genetic variation. Such a scenario could arise if balancing processes, such as sexually antagonistic selection, help preserve variation at sex-linked loci. Alternatively, selection may have acted on standing genetic variation distributed across numerous loci, generating more diffuse allele frequency shifts rather than strong local sweeps. In contrast, the stronger reduction in diversity observed in control males may primarily reflect genetic drift acting in the absence of sustained selection maintaining polymorphism. Together, these results suggest that selection for exaggerated sexual weapons may have altered not only phenotypic evolution but also the genetic diversity on the sex chromosome.

The reduction in diversity on the sex chromosome was more pronounced in males than in females, these results further suggest that sex-specific selective pressures or patterns of inheritance might contribute to shaping genetic variation during experimental evolution. Distinguishing among these non-exclusive mechanisms will require complementary analyses

of allele-frequency trajectories, signatures of selection, and patterns of linkage disequilibrium across the sex chromosome.

Implications for the evolution of trait exaggeration

Our findings challenge and refine several cornerstones of evolutionary theory. First, they demonstrate that directional sexual selection does not inevitably lead to the erosion of genetic variation in exaggerated traits, as often assumed. Instead, selection can interact with genetic architecture and phenotypic plasticity to sustain or even amplify variation, particularly in condition-dependent traits. This has implications for understanding the evolution of sexual dimorphism and the maintenance of biodiversity, as it suggests that strong selection pressures may not always reduce genetic diversity as predicted by classical models.

Second, our study highlights the importance of sexually antagonistic selection as a mechanism for balancing selection. By imposing fitness costs on females, sexual selection on male traits can create a dynamic tug-of-war that prevents the fixation of extreme phenotypes, thereby maintaining polymorphism. This aligns with the growing recognition that intralocus sexual conflict (where males and females share genetic variation but have opposing fitness optima) is a widespread and powerful evolutionary force (Arnqvist & Rowe, 2005; Kaufmann et al., 2023). Such conflicts may explain why many secondary sexual traits exhibit such remarkable variation, even in the face of strong directional selection.

Finally, our results underscore the need for integrative approaches in evolutionary biology. The interplay between genetic variation, phenotypic plasticity, and selection cannot be fully understood in isolation. Future theoretical and empirical work should aim to incorporate these interactions into models of trait evolution, particularly for systems where sexual selection is strong, and traits are highly condition-dependent. This could reveal new insights into the evolution of complexity, the origins of novelty, and the limits of adaptation.

Conclusion: a mechanism for maintaining extreme variation in male weapon size

Combined, our findings reveal a mechanism underlying the evolution of extreme phenotypic variation (Fig. 6). Directional sexual selection, driven by male–male competition, promotes the evolution of exaggerated male weapons by favouring alleles that increase trait size. In contrast, sexually antagonistic selection arising from fecundity costs in females prevents the fixation of these same alleles, thereby maintaining genetic variation within the population.

Under this scenario, low, intermediate and high levels of trait expression are maintained within the population. Fitness costs incurred by males bearing small traits may be offset through the production of more fertile daughters, whereas the reproductive advantages of males with exaggerated traits may be counterbalanced by the reduced fertility of their daughters. Phenotypic plasticity can subsequently amplify this standing genetic variation and blur the boundaries between intermediate and extreme phenotypes, resulting in the broad distributions of trait values observed in exaggerated secondary sexual traits. Similar patterns have been reported in other systems. For example, in the broad-horned flour beetle and in the bulb mites, selection favouring increased male weapon size resulted in reduced lifetime fecundity in females, whereas selection against weapon exaggeration increased female fecundity (Harano et al., 2010; Okada et al., 2021). More broadly, our study highlights how the interaction between genetic conflict, environmental sensitivity and directional selection can be a powerful mechanism generating extreme phenotypic variation in exaggerated secondary sexual traits.

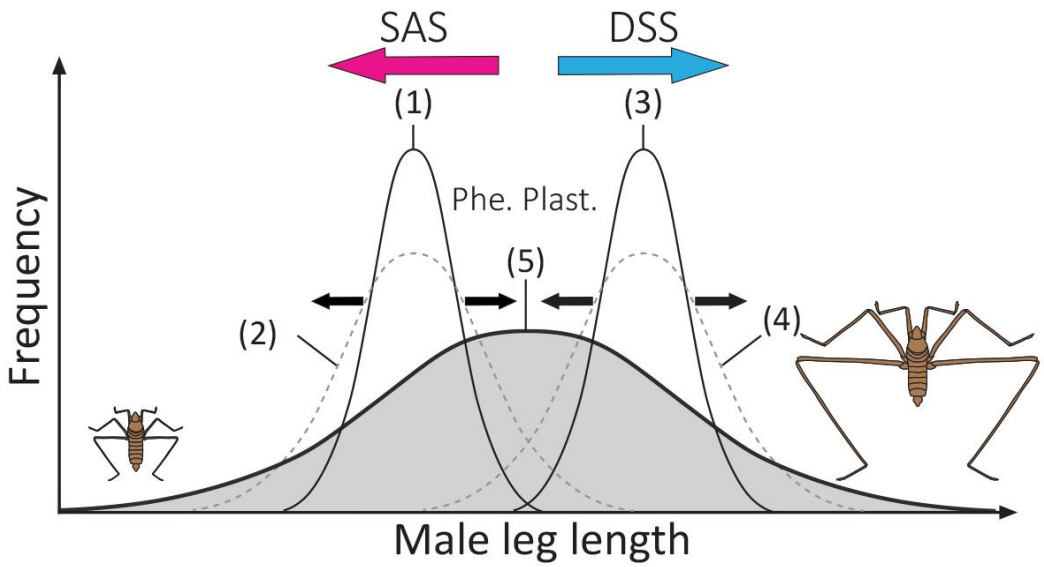

Figure 6. A mechanism underlying the evolution of extreme variation in exaggerated secondary sexual traits. (1) In the absence of directional sexual selection and sexual conflict, exaggerated traits are not expected to evolve, and phenotypic variation remains narrow. **(2)** Under these conditions, phenotypic plasticity alone (black arrows in 1) cannot generate either trait exaggeration or extreme variation. **(3)** Directional sexual selection alone can drive the evolution of exaggerated trait values (blue arrow), but phenotypic variation is expected to remain limited. **(4)** In this scenario, phenotypic plasticity may increase variation slightly (black arrows in 3) but cannot account for the extreme variation observed in many sexually selected traits. **(5)** The interaction between directional sexual selection (blue arrow), sexual conflict (red arrow), and phenotypic plasticity (black arrows) generates the extreme phenotypic variation characteristic of exaggerated sexually selected traits.

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Supplementary figures

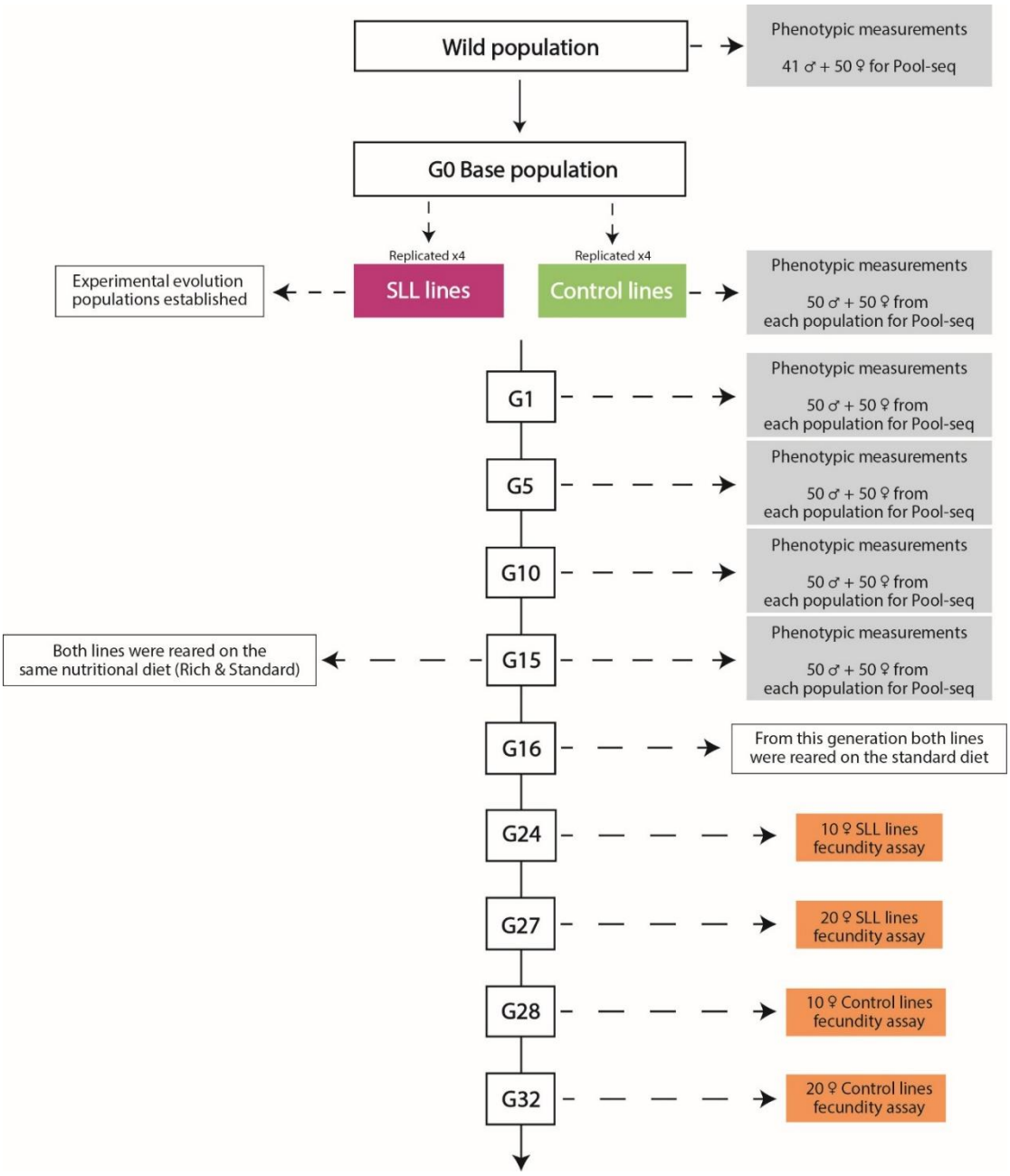

Figure S1. Schematic overview of the experimental design. Shown are the generations when sampling for resequencing and phenotypic assays were performed (grey boxes). Also shown are the generations at which fecundity assays were performed (orange boxes).

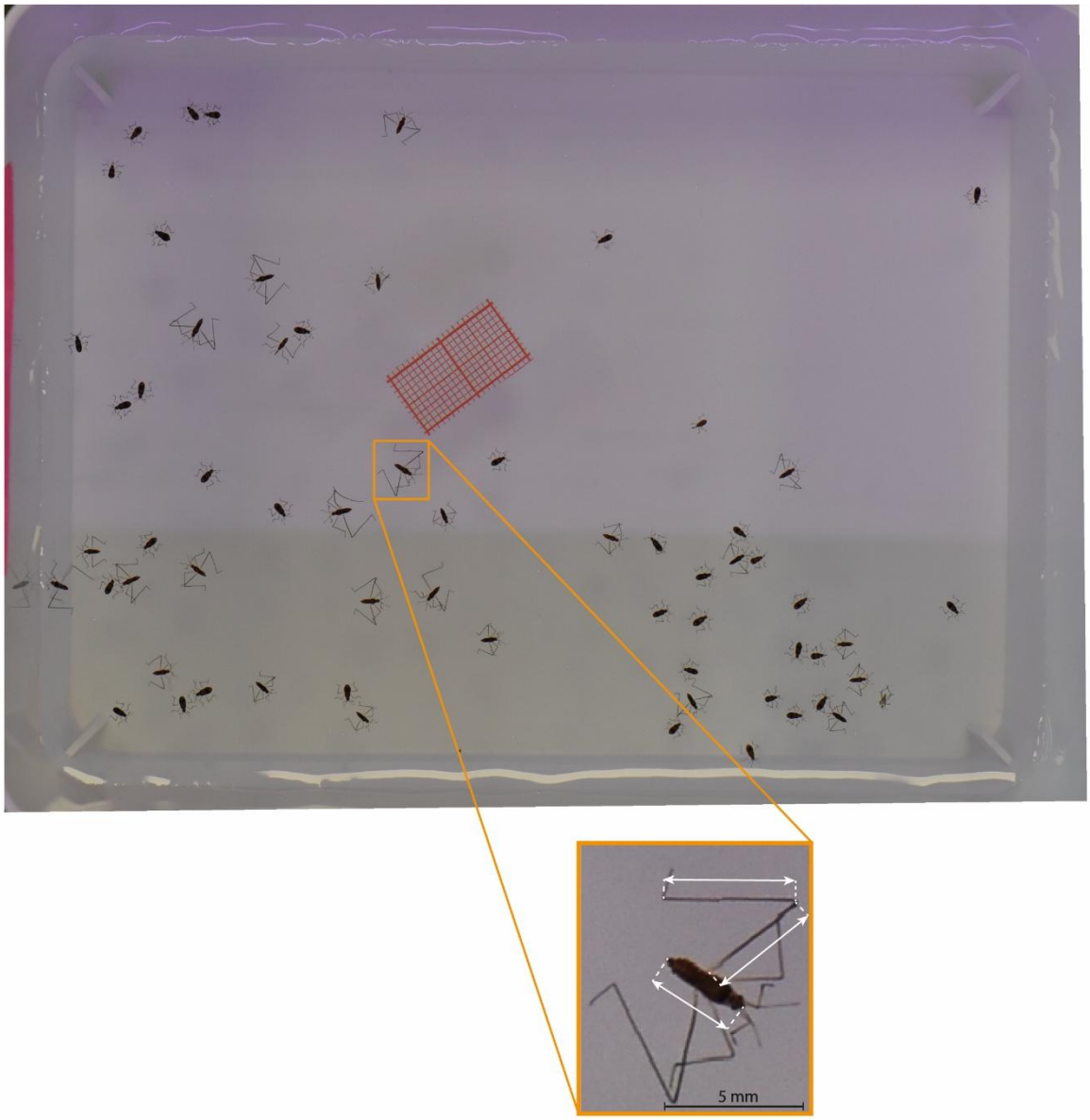

Figure S2. Overview of the phenotypic assay. The orange box represents the scale at which the individuals were measured, and the arrows represent the landmarks used to measure body size, tibia and femur length.

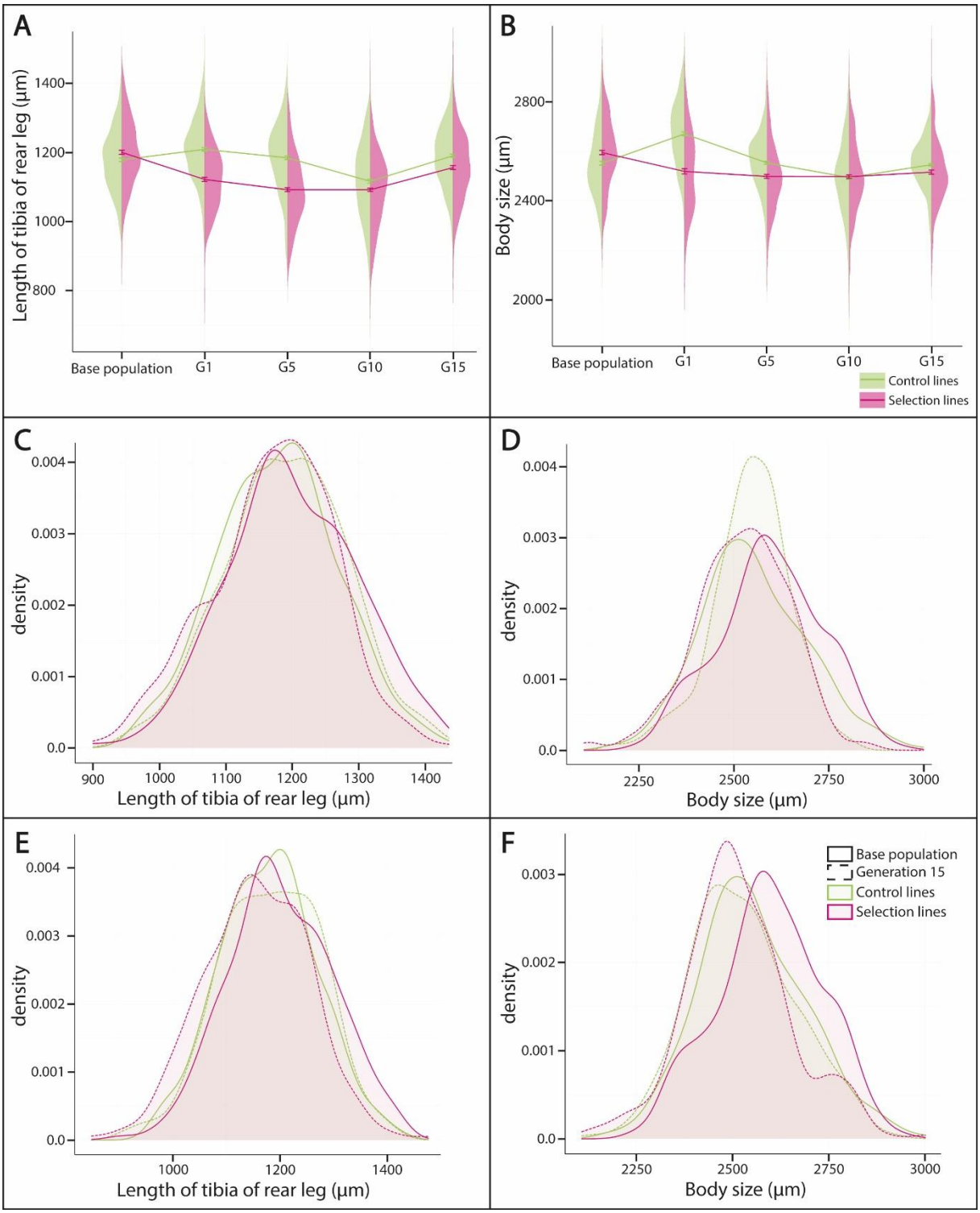

Figure S3. (A) evolution of the length of the rear leg in females, the mean ± standard error is represented. (B) Evolution of the body length in females, the mean ± standard error is represented. (C) and (D) distributions of the length of the rear tibia and body at G15 in the *ad libitum* diet, (E) and (F) distributions of the length of the leg and body at G15 in the standard diet. Green represents the control lines, and purple the selection lines. Plain lines represent the base populations, and the dashed lines represent generation 15.

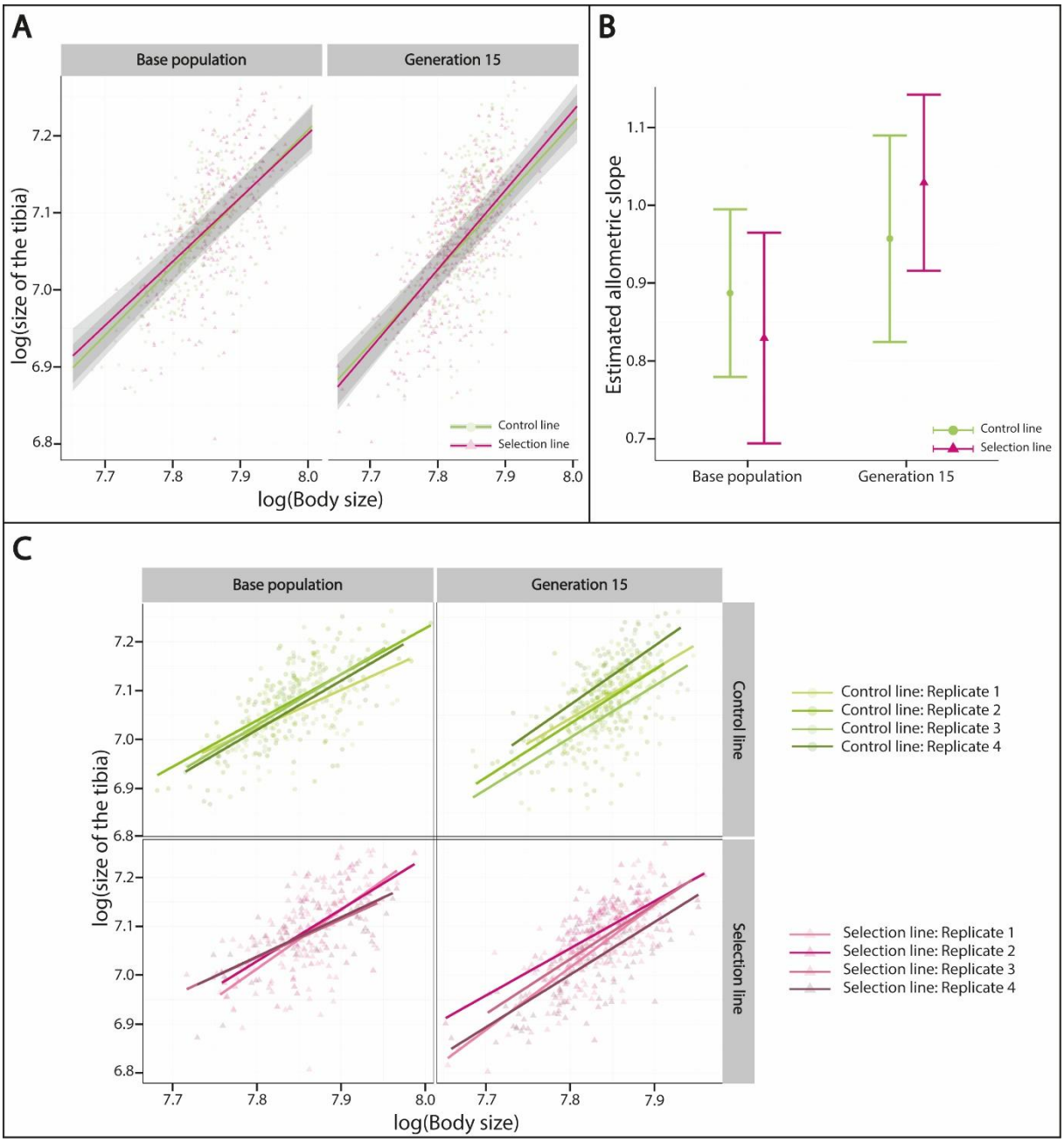

Figure S4. (A) Scaling relationships of log-transformed data between rear tibia and body sizes estimated in females fed on a rich diet from the two lines from the base population and generation 15. (B) Allometric slopes of log-transformed data between rear leg and body lengths estimated in females fed on a rich diet from the two lines from the base population and generation 15, bars represent 95% confidence intervals. Control lines (green and circle), selection lines (purple and triangle). (C) Scaling relationships of log-transformed data between rear tibia and body sizes estimated in females fed on a rich diet from the two lines from the base population and generation 15 for each replicate.

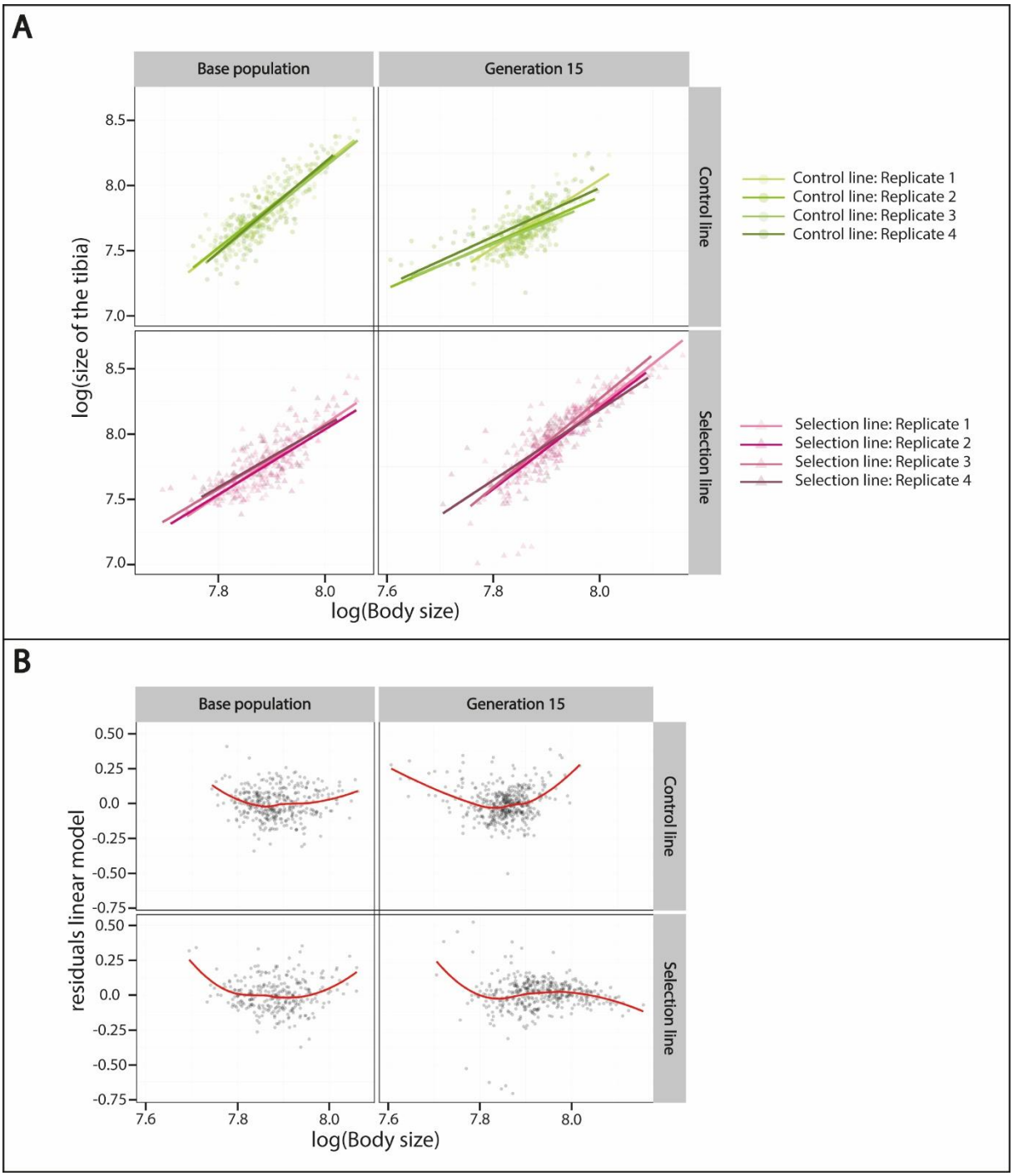

Figure S5. (A) Scaling relationships of log-transformed data between rear legs and body sizes estimated in males fed on a rich diet from the two lines from the base population and generation 15 for each replicate. (B) Residuals of the linear mixed-effects model for males.

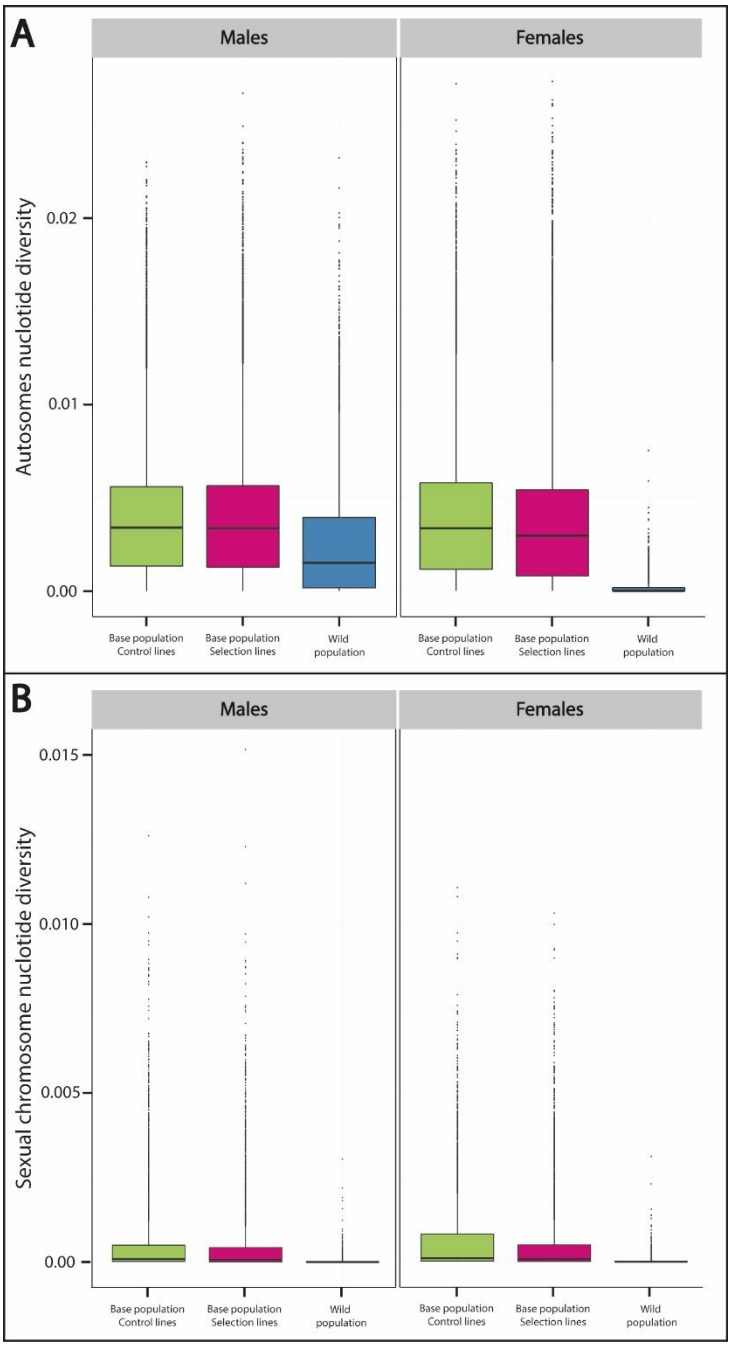

Figure S6. Nucleotide diversity (π) estimated in males and females from the wild population and from individuals of the base population for both lines (A) Nucleotide diversity of autosomes. (B) Nucleotide diversity of the sexual chromosome.

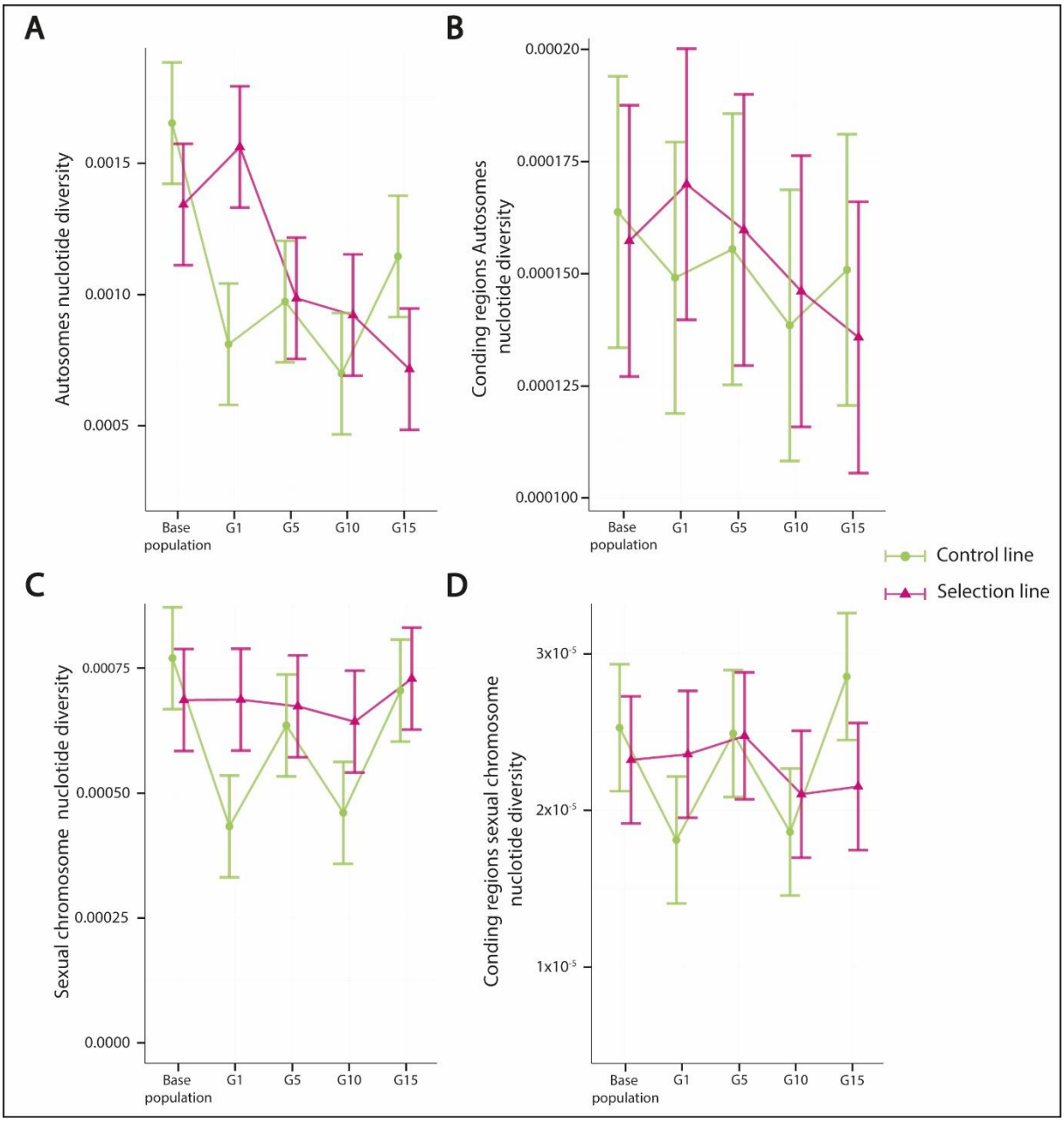

Figure S7. Nucleotide diversity (π) estimated in females from the base population, generation 1, generation 5, generation 10, and generation 15 for both lines. Bars represent 95% confidence intervals. Control lines (green and circle), selection lines (purple and triangle). (A) Nucleotide diversity of autosomes. (B) Nucleotide diversity of autosomes' coding regions. (C) Nucleotide diversity of the sex chromosome. (D) Nucleotide diversity of the sex chromosome's coding regions.

Conclusion

Although this thesis provides new insights into the evolutionary dynamics of exaggerated sexual weapons, several important questions remain unresolved and provide exciting directions for future research. The genomic dataset generated during this thesis offers the opportunity to investigate the genetic basis of the observed phenotypic changes. While the present analyses focused primarily on genome-wide patterns of nucleotide diversity, ongoing analyses aim to identify genomic regions responding to experimental selection through the detection of divergent SNPs, changes in allele frequencies, and additional population genomic statistics, including measures of genetic differentiation genome-wide and on regions containing divergent SNPs. These analyses will help determine whether the phenotypic divergence observed between the experimental lines is associated with localised genomic responses or with changes across the genome.

A particularly interesting question concerns the relationship between genomic diversity and genes involved in nutritional pathways. The expression of exaggerated rear legs is highly condition-dependent, comparing patterns of genetic diversity and allele frequency change in genes associated with nutrition, metabolism, or growth with the rest of the genome could provide valuable insight into the mechanisms maintaining phenotypic variation. A list of nutrition-responsive genes has already been generated in our research group, providing a valuable resource to investigate this. If genes contributing to condition retain higher levels of genetic diversity under sustained directional selection, this could be consistent with predictions of the genic capture model, in which condition-dependent sexual traits capture the genetic variance underlying overall condition (Rowe & Houle, 1996).

Beyond DNA sequence variation, future work should also investigate how genetic variation translates into phenotypic differences. In particular, transcriptomic approaches such as RNA sequencing would determine whether variants identified in candidate genes influence patterns of gene expression during leg development. Combining genomic and transcriptomic data would provide a more complete understanding of the molecular mechanisms underlying weapon exaggeration.

More broadly, integrating experimental evolution with functional genomics represents a promising framework for understanding how standing genetic variation, phenotypic plasticity, and ecological processes jointly contribute to the maintenance of exaggerated sexually selected traits. This approach offers the opportunity to evaluate the relative importance of potentially complementary mechanisms proposed to explain the persistence of phenotypic variation under strong directional selection.

Overall, this thesis demonstrates that prolonged directional selection can rapidly modify weapon size and allometric relationships while substantial phenotypic variation is maintained. Although no evidence of viability costs was detected under the experimental conditions used here, the combination of phenotypic, ecological, and genomic approaches highlights the complexity of the processes shaping exaggerated sexual traits. By coupling experimental evolution with ongoing population genomic analyses and future functional genomic approaches, this work provides a foundation for disentangling the mechanisms maintaining phenotypic variation in an exaggerated sexually selected.

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Annexes

During my thesis, we also published the work that I have done during my M2 internship on sexual conflict in *Gerris odontogaster*: Claudia Pruvôt, David Armisén, Pascale Roux, Göran Arnqvist, Locke Rowe, Arild Husby, Abderrahman Khila, A shared developmental genetic basis for sexually antagonistic male and female adaptations in the toothed water strider, Evolution Letters, Volume 9, Issue 1, February 2025, Pages 13–23, https://doi.org/10.1093/evlett/qrae056

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Letter

A shared developmental genetic basis for sexually antagonistic male and female adaptations in the toothed water strider

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Abstract

Sexual conflict can drive the divergence of male and female phenotypes and cross-species comparative analyses have documented patterns of correlated evolution of sex-specific traits that promote the evolutionary interests of the sexes. However, male–female coevolution can be highly dynamic, particularly if the male and female traits share an underlying genetic program. Here, we use water striders, a well-studied model system for sexually antagonistic coevolution, and ask whether sex-specific phenotypic adaptations covary across populations and whether they share a common developmental genetic basis. Using comparative analyses both at the population and species levels, we document an association between a derived male mate-grasping trait and a putative female anti-grasping counteradaptation in the toothed water strider *Gerris odontogaster*. Interestingly, in several populations where males have partly lost their derived grasping trait, females have also reduced their antigrasping adaptation. We used RNAi to show that these male and female traits are both linked to a common developmental genetic program involving Hox- and sex-determination genes, despite the fact that they are different structures on different abdominal segments. Our work illustrates the dynamic nature of sexually antagonistic coevolution and suggests that the pleiotropic nature of developmental genetic programs can blur the distinction between inter- and intralocus genetic conflict.

Keywords: sexual conflict, coevolution, evolution of sex, development

Lay Summary

The evolutionary interests of males and females often diverge, particularly over mating interactions. This coevolutionary pattern leads to the evolution of adaptations and counteradaptations that further the evolutionary interest of each sex. How the phenotypic divergence of the sexes emerges at small time scales and despite the shared genome is not fully understood. We address these questions in the toothed water strider, *Gerris odontogaster*, a classical model for the study of sexual conflict. By analyzing multiple populations and species, we uncover strongly correlated evolution between male abdominal teeth that act to grasp the female and a counteradaptation in the females consisting of a concealed proctiger that is the target of the male trait. We also show, through manipulating gene expression, that the male and the female traits share, at least partly, an underlying developmental genetic program containing both Hox- and sex-determination genes. This study paves the way to testing the extent of overlap between intra- and interlocus genetic conflict.

Introduction

Males and females share most of their genomes, yet their evolutionary interests often diverge which generates evolutionary conflict (Arnqvist & Rowe, 2005; Bonduriansky & Chenoweth, 2009; Parker, 2006). We distinguish between intralocus and interlocus sexual conflict (Schenkel et al., 2018). The former occurs when males and females have different optimal values for a shared phenotypic trait, which is manifested at a genetic level as different alleles at a given locus being favored in males and females. Examples include height in humans (Stulp et al., 2012), age at maturity in salmons (Mobley et al., 2021), and body size in beetles (Berger et al., 2016; Kaufmann et al., 2021). A symptomatic pattern of intralocus sexual conflict is a negative intersexual genetic correlation for fitness (Connallon & Matthews, 2019). In contrast,

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interlocus sexual conflict occurs when the optimal outcome of intersexual interactions differs in males and females. This can lead to the evolution of suites of sexually dimorphic traits encoded by different loci in males and females that affect a given outcome, through a process known as sexually antagonistic coevolution (Arnqvist & Rowe, 2005). Potential examples include the evolution of reproductive traits in seed beetles (Dougherty et al., 2017; Rönn et al., 2007), mollusks (Koene & Schulenburg, 2005), millipedes (Sloan et al., 2023), and guppies (Evans et al., 2011), as well as mate-grasping traits in diving beetles (Bergsten & Miller, 2007; Bergsten et al., 2001) and water striders (Arnqvist & Rowe, 2002a, b).

Although sexually antagonistic coevolution is thought to be a major driver of diversification and speciation (Arnqvist et al., 2000; Parker & Partridge, 1998; Rice et al., 2005), we actually know very little about its genetic basis (Pennell et al., 2016; Schenkel et al., 2018). This is unfortunate, because intra- and interlocus sexual conflict may not be entirely distinct if loci with effects on sex-specific traits involved in interlocus sexual conflict have pleiotropic effects with fitness consequences in the opposite sex (Geeta Arun et al., 2022; Mokkonen et al., 2016). If sexually dimorphic traits that are undergoing correlated evolution, to some degree, have a shared genetic basis, the genetic correlation between male and female traits could significantly affect the predicted dynamics of sexually antagonistic coevolution (Pennell et al., 2016).

In water striders (Gerridae), a monophyletic group of semiaquatic Heteroptera, conflict over mating is widespread and is known to drive escalation of both grasping traits in males and antigrasping traits in females (Arnqvist, 1997; Arnqvist & Rowe, 2002a, b). In this sexually antagonistic coevolution, males are favored to mate repeatedly while multiple mating can be superfluous and costly for females (Arnqvist & Rowe, 2005; Rowe, 1994). The outcome of male–female interactions is determined by premating struggles, during which the male strives to secure an anterior and a posterior grasp of the female, while the female tries to dislodge the male (Arnqvist & Rowe, 2005). Sexually antagonistic traits that affect the outcome of struggles include modifications of the appendages or other body parts into claspers in males, and the presence of spines, various integument projections, and modifications of the tip of the abdomen in females that make it more difficult for males to grasp females posteriorly during mating (Arnqvist, 1989, 1997; Arnqvist & Rowe, 2002b; Crumière et al., 2019; Rowe et al., 2006). Phenotypic manipulation and behavioral studies have demonstrated the function of male and female sexually antagonistic traits during premating interactions (Arnqvist, 1989; Arnqvist & Rowe, 1995; Crumière et al., 2019; Han & Jablonski, 2009; Khila et al., 2012; Ronkainen et al., 2005), and a few studies have identified important genes required for shaping these traits during development (Crumiere & Khila, 2019; Khila et al., 2012).

Phylogenetic comparative analyses have revealed patterns of correlated evolution between the sexes under the influence of sexual conflict (Perry & Rowe, 2018). In water striders, Arnqvist and Rowe (2002a, b) showed that the primary correlated changes are manifested in the shape of genital segments in both sexes, which together determine the posterior grip during struggles, and variation in these sexually antagonistic traits correlates with variation in the economics of mating. Population-level analyses also revealed that ecological factors, such as food resources, predation, or population density, impact the patterns of antagonistic coevolution of the sexes by altering the costs and benefits of mating (e.g., Arnqvist, 1994; Perry & Rowe, 2012; Rowe, 1994). In theory, the dynamics of sexually antagonistic coevolution in terms of escalation and de-escalation may thus be affected by both external and internal factors. First, environmental variation may act as a driver of sexually antagonistic coevolution and phenotypic divergence between males and females (Perry & Rowe, 2012; Rowe et al., 2018). Second, the evolution of sexually antagonistic adaptations will affect their relative efficacy, and this in itself can generate escalation and de-escalation (Parker, 1979; Rowe et al., 2005). Third, the genetics of interlocus sexual conflict can itself contribute to the dynamics of sexually antagonistic coevolution. In particular, between-sex pleiotropic constraints can act to generate cycles of escalation and de-escalation (Pennell et al., 2016).

It is therefore important to assess the genetic and developmental basis for sexually dimorphic traits involved in sexually antagonistic coevolution. Here, we focus on a classic water strider species in this context—the toothed pondskater *Gerris odontogaster*. We first use comparative analyses at the micro- and macro-scale, to assess whether and how a well-known male grasping trait (consisting of two extensions of the seventh abdominal tergite) is matched by a female counteradaptation. We study populations exhibiting significant variation, including the remarkable near loss of the male grasping trait. Using experimental manipulation of gene expression, we then link covariation in male and female traits to the role of a developmental genetic program containing both Hox- and sex-determination genes.

Materials and methods

Study species

The various populations of *G. odontogaster* used in this study, their geographic locations, and source are described in Supplementary Table S1. Experimental populations of *G. odontogaster* from Sweden and *G. buenoi* from Canada were reared in water tanks in a room maintained at 28 °C with a relative humidity between 40% and 50%, under an artificial photoperiod of 14 hr of daylight. Animals were fed daily with frozen crickets. Females lay eggs on floating Styrofoam pieces that were removed weekly and put into new water tanks to let the eggs hatch.

Phenotyping

Males and females from each species and population were photographed using a microscope, and then measured using software associated with the microscope. For males, the length of the male abdominal processes (MAPs) and of the body were measured from a lateral view of the animal (see Supplementary Figure S1). For females, the visible part of the proctiger and the plate that recovers it were measured from a dorsal view (see Supplementary Figure S2).

Phylogenetic reconstruction and PGLS analysis

A transcriptomic-based phylogenetic reconstruction approach was used as described in Armisen et al. (2022). Transcriptomic data were generated using Illumina sequencing for the freshly collected populations of *G. odontogaster* (excluding museum samples) as well as for *G. buenoi*, *G. lacustris*, *G. incognitus*, and *G. marginatus*. Transcripomes were assembled using Trinity (Grabherr et al., 2011) and used to define clusters of orthologous genes using OrthoFinder (Emms & Kelly, 2019). After testing for stationary, reversible, and homogeneous consistency (Naser-Khdour et al., 2019), a final list of 5,658 gene clusters was retained for phylogenetic reconstruction using IQ-TREE (Nguyen et al., 2014). To further test the relationship between the three *G. odontogaster* populations, an additional likelihood mapping analysis was performed to determine the phylogenetic relationship between the

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three *G. odontogaster* populations (Supplementary Figure S3). To do so, we used IQ-TREE (option: -n 0 -lmap 10,000 -m TEST -bsam GENESITE) to test possible topologies between: outgroups (other *Gerris* species), *G. odontogaster* Swedish, *G. odontogaster* France, and *G. odontogaster* Italy (Supplementary Figure S3). Phylogenetic least-squares regression was performed with ape v. 5.8 and in R v 4.3.3 (R_Core_Team, 2024).

RNA interference against *Abd-B* and *dsx*

For this experiment, nymphs from the fourth and early fifth instars of the Swedish population (largest MAPs) were injected with double-stranded RNA (dsRNA) of *Abdominal-B* and *doublesex*, separately, to induce degradation of transcripts corresponding to these genes. The cloning of *doublesex* and *Abdominal-B* was done by PCR based on sequences of *G. odontogaster* that were extracted from a full transcriptome database hosted at IGFL Lyon (https://gerromorpha.igfl.ens-lyon.fr/equipes/a.-kh-ila-developmental-genomics-and-evolution/). Primers were designed based on these sequences using Primer-BLAST software. The cloning of *Abd-B* and *dsx* fragments was carried out using standard PCR and cloning techniques. Primers and gene sequences can be retrieved in GenBank under the following accession numbers: *Abdominal-B*: PQ283678, *doublesex*: PQ283677.

In vitro transcription of *dsx* dsRNA

Templates flanked by T7 promoters were synthesized by PCR using the primer sequences by Lynch and Desplan (Lynch & Desplan, 2006). A total of 1 µg of each T7 PCR template was used to synthesize double-stranded RNA of *dsx* and *Abd-B* (Lynch & Desplan, 2006). In total, 10 µl of 10× transcription buffer, 10 µl of 10 mM dNTPs, 1 µl of T7 RNA polymerase Plus (200 Units/µl), 1 µl of RNaseOUT, and a volume of nuclease-free water to obtain a final volume of 100 µl. The reaction was incubated at 37 °C overnight: 2 µl of DNase (1 Unit/µl) was added to the reaction, which was incubated at 37 °C for 10 min. Subsequently, the dsRNA preparation was purified using the RNeasy Mini Kit (Qiagen). Injection buffer (Rubin & Spradling, 1982) 10× was added (10% of total volume). Finally, the dsRNA solution was filtered with Syringe Driven Filter Unit Cat and was centrifuged at 5,000 rpm for 30 s.

Injection of dsRNA of *dsx* and *Abd-B* into nymphs of *G. odontogaster*

Individuals from the fourth instar to the early fifth instar of the Swedish population of *G. odontogaster* were used for this experiment due to the fact that the formation of MAPs begins 65 hr after the fifth molt. Both sexes were injected to test whether or not the genes had a sex-specific function. Animals were anesthetized with carbon dioxide for 15–20 s and then were injected between the sixth and the seventh abdominal segments with a 0.7 µg/µl dsRNA solution for *dsx* and 0.5 µg/µl for *Abd-B*. Each individual was injected with approximately 0.5 µl of this solution. Animals injected with the same volume of injection buffer 1× were used as negative controls to ensure that the trauma caused by injection did not induce a change of phenotype. Injections were delivered *via* quartz needles with filaments using a Zeiss Stereo Discovery V8 binocular magnifier. After injection, the animals were kept in separate water tanks and were fed on crickets twice a day until adulthood. After the injection of *doublesex*, males and females were kept separated in case the depletion of *dsx* transcripts had an effect on sex determination. When they became adults, individuals were sacrificed in ethanol, screened for phenotypes, and photographed using a Keyence VHX 7000 microscope. The experiment was terminated when at least 10 male adults per condition survived and had a modified phenotype.

Fluorescence staining of MAPs

Mid-fifth instar male nymphs were dissected and the tergite of the seventh abdominal segment with developing MAPs recovered. The tissue was fixed for 30 min in 4% paraformaldehyde, then washed five times in phosphate-buffered saline (PBS) containing 0.05% Tween-20 (PTW). Tissues were blocked for 1 hr in an antigen-blocking solution containing 0.1% bovine serum albumin, 1% normal goat serum, 1× PBS, and 0.05% Tween-20. The tissues were then incubated with mouse acetylated alpha-tubulin antibody overnight at 4 °C. The tissues were washed five times, 10 min each, with PTW and incubated 2 hr with an antimouse antibody and phalloidin (actin dye). Tissues were washed five times, 10 min each, with PTW and then 5 min in 1× PBS containing 30% glycerol (in PBS). Tissues were incubated for 10 min in 1× PBS, 50% glycerol containing DAPI (4′,6-diamidino-2-phenylindole). Finally, the tissues were mounted on slides in 8% glycerol. Images were captured using a Zeiss LSM 710 confocal microscope.

Data analyses

Statistical analyses were conducted using R (v 4.2.2). The ratio of MAPs size/body size was compared between the males of the different *G. odontogaster* populations. The effect of population on this ratio was tested through a one-way analysis of variance. The normal distribution and homoscedasticity of the residuals were tested, respectively, through a Shapiro–Wilk test and a Bartlett test, and a graphical inspection of residuals was performed. When a significant difference was detected a Tukey post-hoc test was used to determine which populations differed. The same procedure was used to determine the impact of population and/or species on the ratio of proctiger size/plate size between females.

Results

Correlated evolution of a male adaptation and a putative female counteradaptation

Males of *G. odontogaster* use their MAPs (Figure 1A) to grasp the proctiger of the female during premating struggles and MAPs play an important role in increasing the mating rate by providing posterior grasp (Arnqvist, 1989). Females with a proctiger that is harder to clasp by MAPs should lower potential fitness costs associated with superfluous matings and, therefore, be favored by selection. We here hypothesize that a concealed female proctiger represents an adaptation in females to counter the effect of MAPs. This tenet is based on (a) comparative analyses in this clade showing negatively correlated evolution between male clasping ability and the accessibility of the proctiger, which affects the mating rate (Arnqvist & Rowe, 2002b), (b) experimental manipulations showing that a less accessible tip of the abdomen makes it easier for females to dislodge males (Arnqvist & Rowe, 1995; Ronkanen et al., 2005), and (c) studies of other semiaquatic heteropterans showing that concealed genitalia represents a female counteradaptation (Han & Jablonski, 2009; Maroni et al., 2023). Populations of *G. odontogaster* differ considerably in the mean length of MAPs, even at a small geographic scale, which is partly related to local population density and predation (Arnqvist, 1992b, 1994). Remarkably in some populations, males have significantly reduced MAPs and only show rudiments of this structure (Figure 1A–D). This was first reported in Mongolia (Lansbury, 1966; Lundblad, 1934), and

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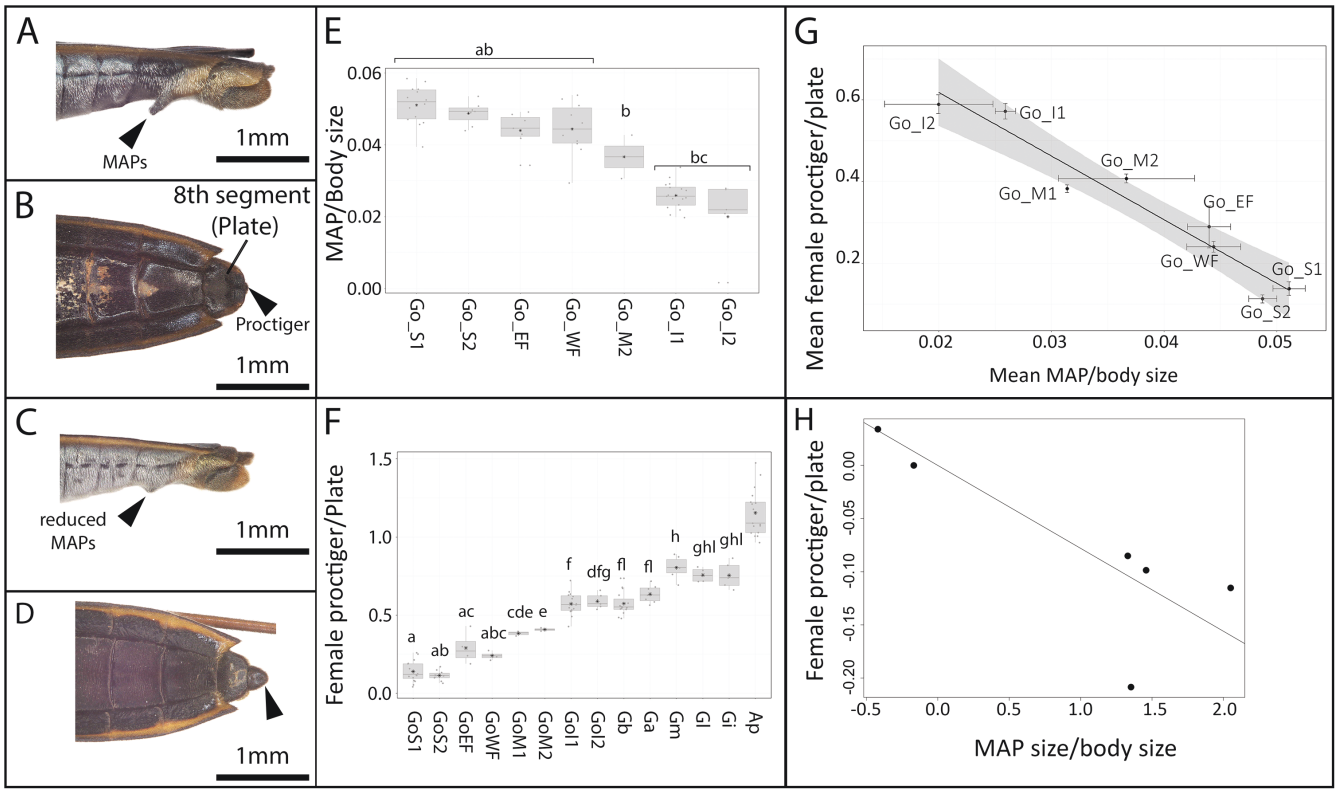

Figure 1. Phenotypic variation in the size of MAPs on the seventh abdominal segment and the state of proctiger (eighth abdominal segment) concealment in *G. odontogaster*. (A, B) A male and a female of a Swedish population. (C, D) A male and a female of an Italian population. (E) MAPs size across seven populations of *Gerris odontogaster*. Note that one Mongolian population is represented by a single male and therefore not included in MAPs analysis. Letters denote significant ($p < 0.05$) differences between populations in Tukey post-hoc tests. (F) Proctiger concealment across eight populations of *Gerris odontogaster* and other Gerridae (see Supplementary Table S1 for description of populations). Go: *Gerris odontogaster*; S1 and 2 are two distinct populations from Sweden; EF: Eastern France; WF: Western France; I1 and 2: two distinct populations from Italy; M1 and M2 are two distinct populations from Mongolia. (G) Plot of the mean MAPs size and proctiger concealment in the eight populations of *G. odontogaster*. Error bars correspond to SE, line represents a linear regression and shaded area the 95% CI. (H) Phylogenetic generalized least square regression of proctiger concealment on MAPs size and phylogenetically independent contrast values across three populations of *G. odontogaster* (Sweden, France, and Italy) and four additional species of *Gerris*. Abbreviations: Gb: *Gerris buenoi*; Ga: *Gerris argentatus*; Gm: *Gerris marginatus*; Gi: *Gerris incognitus*; Gl: *Gerris lacustris*; Ap: *Aquarius palludum*.

later found in northern Italy (Tamanini, 1979; Wagner, 1958) and Slovenia (Tamanini, 1979). Individuals from these populations were even given subspecific status as *G. odontogaster brevispinis* (Lundblad, 1934), although such populations are thought to represent local forms rather than a taxonomic unit (Andersen & Chen, 1993). To test our predictions that the proctiger in females is a counteradaptation to male MAPs, we phenotyped males and females from in total eight populations of *G. odontogaster* (Figure 1E and F, also see Supplementary Table S1 for population origin; Supplementary Table S2 for raw measurements). First, we phenotyped two Swedish *G. odontogaster* populations where males have fully formed and functional MAPs (Figure 1A and E). In these populations, the proctiger of the females is almost fully concealed under a dorsal plate consisting of a modified eighth abdominal segment (Figure 1B and F). Second, we phenotyped two Italian populations (a contemporary population collected in 2022 and a population collected in 1947) and two Mongolian populations where males have rudimentary MAPs (Figure 1C–F) (Lansbury, 1966; Lundblad, 1934). Here, we predicted that rudimentary MAPs should be associated with relaxed selection on the concealment of the females' proctiger. Consistent with this prediction, the females from the Italian populations have a proctiger that is about four times more exposed compared to *G. odontogaster* from Sweden (Figure 1F). The same result was seen in the Mongolian populations, as females have a proctiger that is some three times more exposed than the Swedish populations (Figure 1F). Third, we phenotyped *G. odontogaster* from Western and Eastern France, where males have MAPs that are of intermediate size between the Swedish and the Italian populations (Figure 1E). Interestingly, females also exhibited an intermediate state of proctiger concealment (Figure 1F). For comparison, we also phenotyped a population of *G. buenoi*, a closely related species to *G. odontogaster*, where males lack MAPs altogether. Again, the females of *G. buenoi* have an exposed proctiger (Figure 1E and F). A closer inspection showed that the degree of proctiger concealment is primarily defined by the size of the eighth abdominal segment, which consists of a dorsal plate on top of the proctiger. In *G. odontogaster* from Sweden, this plate is significantly larger and covers the proctiger, whereas this segment is narrower in *G. odontogaster brevispinis* from Italy and in other *Gerris* species allowing the proctiger to remain exposed (Figure 1B–D). The intersexual correlation between the mean length of MAPs in males (the ratio between MAP length and body size) and average proctiger concealment in females (the ratio between proctiger protrusion and the length of the dorsal plate) across the eight *G. odontogaster* populations was $r = -0.92$ ($p < 0.001$), providing evidence for negatively correlated evolution across populations (Figure 1G).

Next, we wished to better place the above covariation between MAPs and the state of proctiger concealment across *G. odontogaster* populations in a phylogenetic context. A phylogenetic

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reconstruction, based on the transcripts of 5,658 genes (Armisen et al., 2022), of a sample of *Gerris* spp along with three populations of *G. odontogaster* allowed us to assess the history of sexually antagonistic coevolution and to compensate for the potential effect of phylogenetic dependencies through the use of phylogenetic generalized least squares (PGLS) regression (Rohlf, 2001). The PGLS test of correlated evolution between the length of MAPs in males and proctiger concealment in females (Supplementary Table S3) revealed significant negative correlated evolution ($b = -0.078$, $SE_b = 0.016$, $p = 0.005$). The phylogenetic reconstruction also revealed that *G. odontogaster* from France and *G. odontogaster brevispinis* from Italy are sister populations and that *G. odontogaster* from Sweden is basal to both (Figure 2; Supplementary Figure S3). In addition, this reconstruction placed *G. buenoi* as a sister to all populations of *G. odontogaster* (Figure 2), confirming that the acquisition of MAPs is a derived state in *G. odontogaster*. Interestingly, this reconstruction also showed that the loss of MAPs in *G. odontogaster brevispinis* from the Italian population is a secondary event that followed their acquisition (Figure 2), providing evidence for a de-escalation of sexually antagonistic coevolution. The evolutionary pattern of proctiger concealment is consistent with the state of the MAPs, suggesting that this concealment might be costly and that the ancestral state of proctiger exposure is regained by females when MAPs are lost in males (Figures 1 and 2). These data further strengthen the suggestion that selection on proctiger state in females is related to MAPs and involved in sexually antagonistic coevolution.

Cellular and developmental genetic basis of MAPs

Water striders belong to the Heteroptera suborder with post-embryonic development consisting of five successive molts (Andersen, 1982) (Supplementary Figure S4). Phenotypic differentiation of males and females can only be observed in the genital segments at the fourth and fifth nymphal instars (Supplementary Figure S4). The first signs of MAP specification appear at the fifth, and final, nymphal instar about 65 hr after molting from the fourth instar (Figure 3A and B). The developing MAPs can be observed through the cuticle of the fifth instar nymph after treatment with alcohol (Figure 3A), or by peeling the cuticle of the nymph and uncovering the differentiating preadult integument (Figure 3B). MAPs development starts as a pair of cell populations organized into concentric circles that prefigure their position in the future adult (Figure 3B and C). The lumen of the developing MAPs is particularly rich in cytoskeletal material as revealed by actin and acetylated alpha-tubulin staining (Figure 3D). By the end of the fifth nymphal instar, MAPs extend and can be readily observed in the preadult after removing the nymphal integument (Figure 3C). These cellular and developmental dynamics suggest that the development of MAPs involves cytoskeleton remodeling and morphogenetic processes.

Hox- and sex-determination genes shape MAPs during development

The development of MAPs is highly heritable (Arnqvist, 1989), but the genes involved are not known. We hypothesized, based on the state of knowledge about the role of Hox- and sex-determination genes in establishing differences along the body axis between males and females, that *Abdominal-B*, a posterior Hox gene, and *doublesex* will be required for the development of male and female traits. We therefore used the RNA interference technique to knock down the transcripts of these genes and test their roles in the development of MAPs and proctiger concealment (Supplementary Table S4). Control males from the Swedish population, injected with buffer, were indistinguishable from wild-type males and MAPs were present and fully developed on the seventh

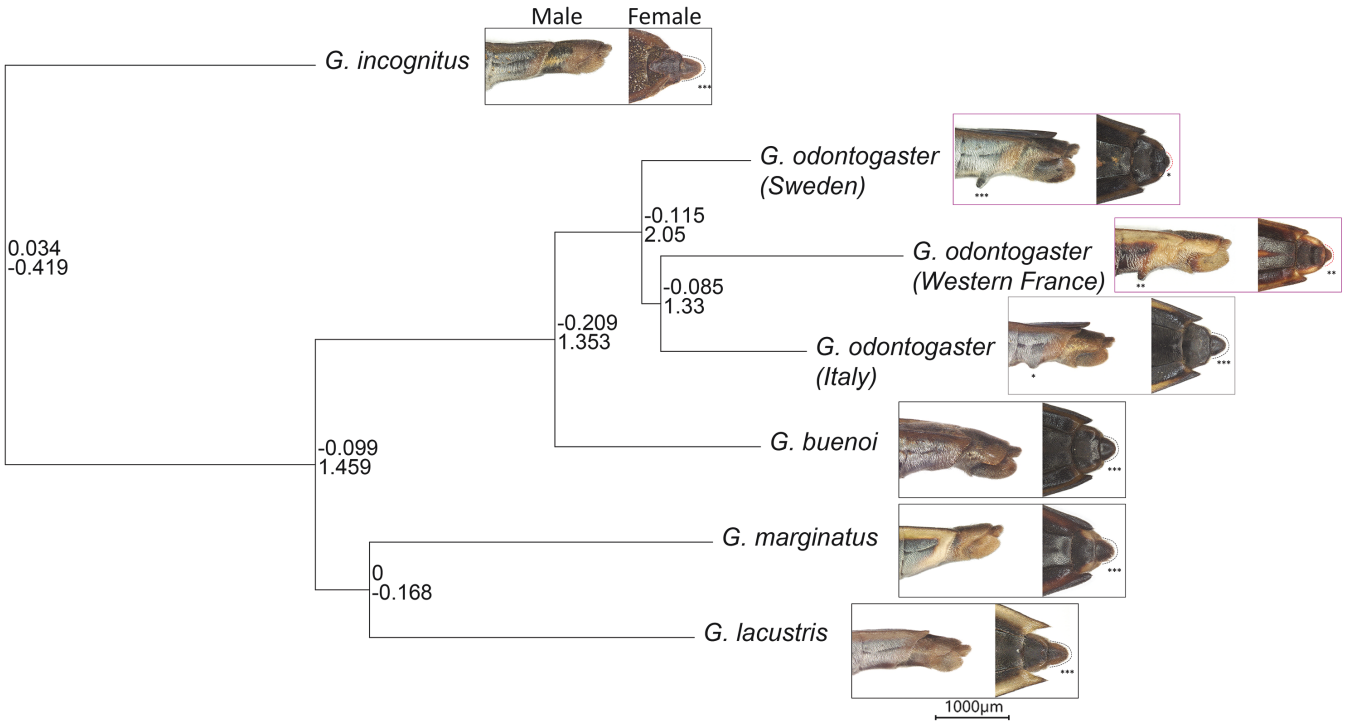

Figure 2. Phylogenetic relationships between three populations of European *G. odontogaster* and a sample of *Gerris* spp. Number of asterisks reflects the size of MAPs and the state of proctiger exposure. Phylogenetically independent contrast values for the male and female traits are indicated in each node.

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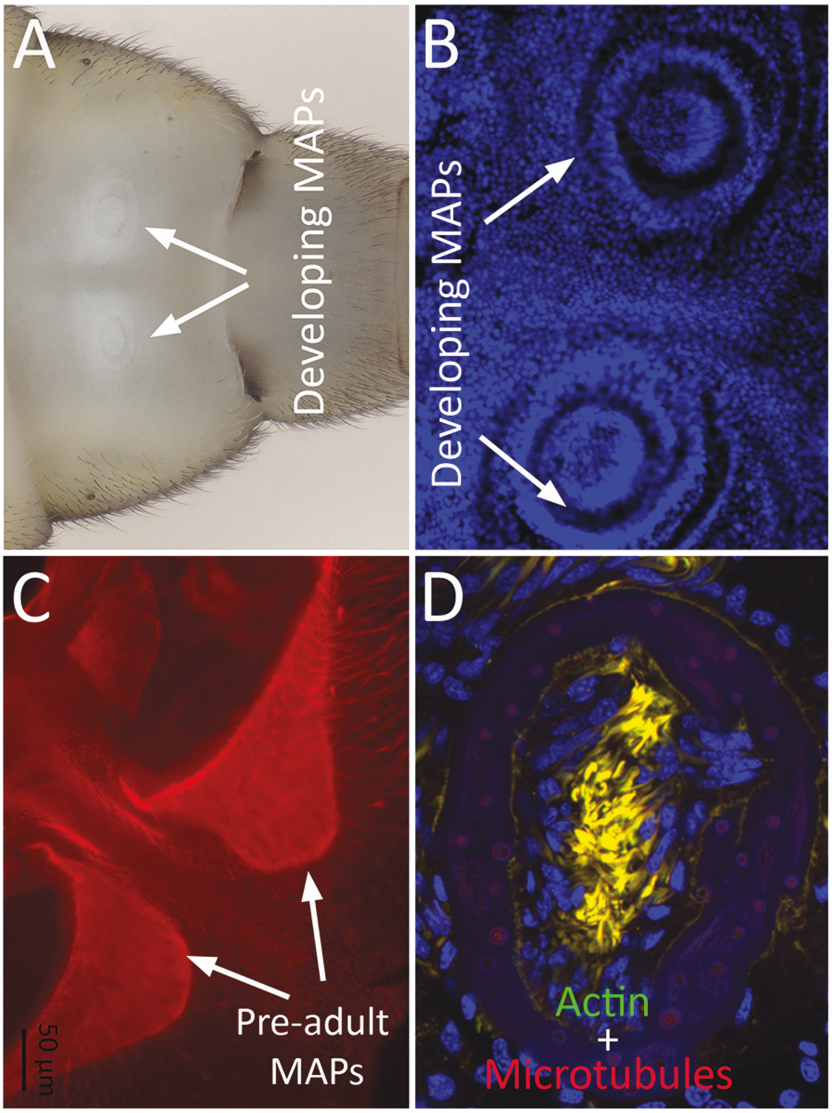

Figure 3. Development of MAPs. (A, B) The first signs of the development of MAPs appear at the middle of the fifth, and final, nymphal instar. (A) Two structures, prefiguring adult MAPs, can be visualized by imaging the nymphs in alcohol. (B) Epithelium of a preadult dissected out of the fifth instar nymph and stained with the nuclear marker DAPI. Developing MAPs consist of two sets of concentric cell populations which extend into full MAPs at the adult stage (C). (D) Actin and acetylated alpha-tubulin staining shows that the lumen of the developing MAPs is rich in cytoskeleton and microtubules. The yellow color results from the overlap of actin (green) and tubulin (red).

abdominal sternite ($n = 7$, Figure 4A and B; Supplementary Table S4). In wild-type and control males, the posterior part of this sternite had the shape of a trough that, we suggest, enables males to extend their genitalia in a more ventral angle in order to better reach females' genitalia during permating struggles and mating (dashed outline in Figure 4B). A total of 15 fourth and early fifth instar males injected with dsRNA targeted against *Abdominal-B* and 13 males injected with dsRNA targeted against *dsx* reached adulthood. Knockdown of both *Abdominal-B* and *doublesex* resulted in the reduction or the complete loss of the MAPs (Figure 4C and E; see Supplementary Table S4 for quantification). In addition, the knockdown of both genes resulted in the disruption of the trough-shaped posterior part of the seventh abdominal sternite, which instead became more linear (dashed outline in Figure 4D and F). This overlap in the effect of *Abdominal-B* and *doublesex* suggests that the two genes interact within a network that shapes male abdominal processes.

Abdominal-B, but not *doublesex*, is necessary for concealing female's proctiger and shaping the genitalia

Control-injected fourth and early fifth instar females were indistinguishable from wild-type females ($n = 7$, Figure 4G and H). A total of 11 females reached the adult stage after the injection of *Abdominal-B* double-stranded RNA. Interestingly, the eighth abdominal segment that covers the proctiger became narrower leaving the female structure exposed ($n = 11$, Figure 4I). This suggests that *Abdominal-B* is part of a genetic mechanism that shapes a female's putative counteradaptation through the concealment of the proctiger. The gonocoxae of wild-type females cover the ovipositor and the gonapophyses (Figure 4H). *Abdominal-B* knockdown resulted in a partial exposure of these structures ($n = 7$), and for two females they were completely exposed (Figure 4J). Adult females resulting from *dsx* knockdown had no clear morphological differences compared to controls ($n = 16$, Figure 4K and

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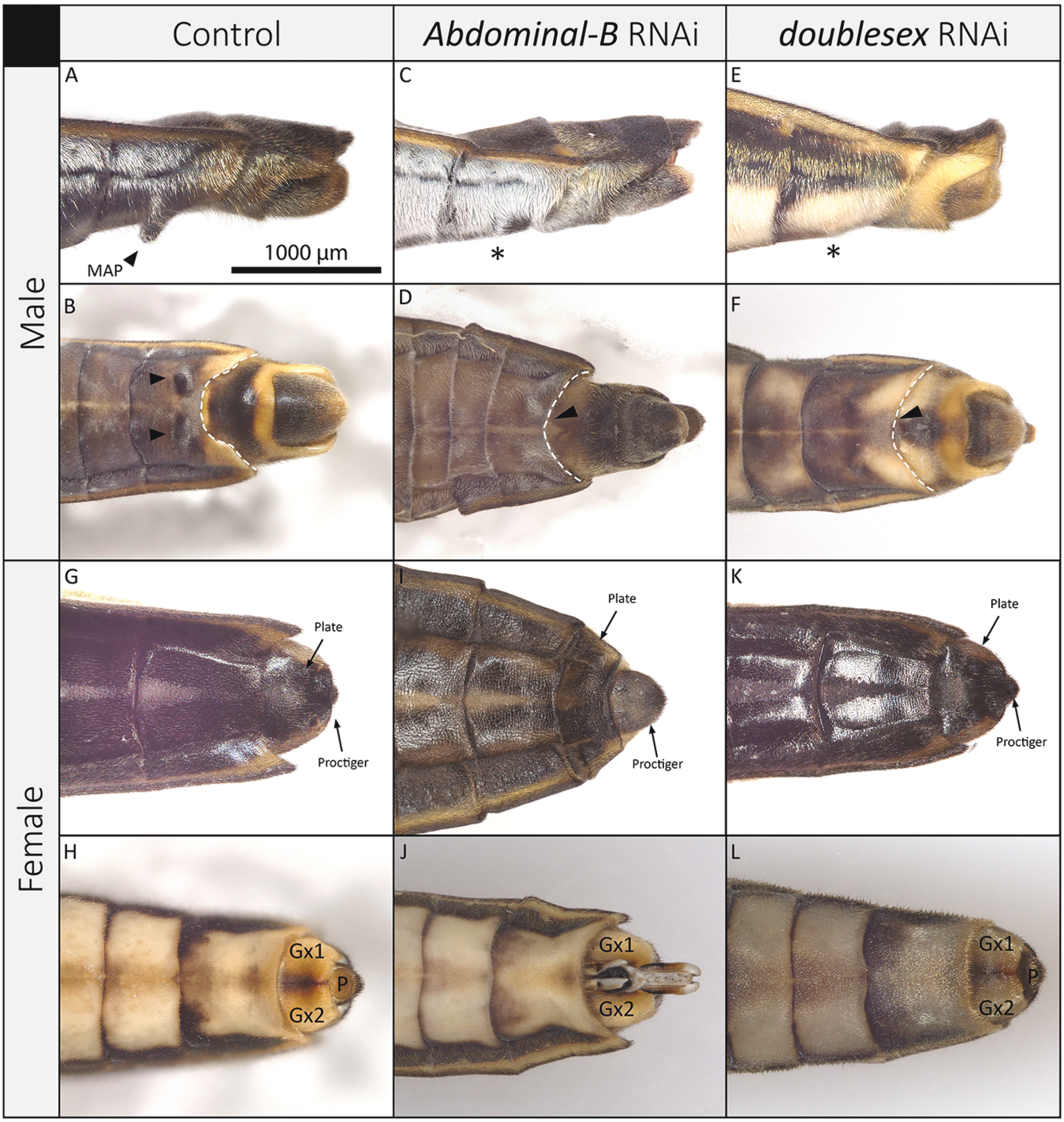

Figure 4. All images represent the Swedish population of *G. odontogaster*. Role of *Abdominal-B* and *doublesex* in the development of MAPs and proctiger concealment. (A) Lateral view of a control male showing fully extended MAPs. (B) Lateral view of a control male showing the trough shape of the seventh abdominal segment (dashed outline). (C, D) *Abd-B* RNAi and (E, F) *dsx* RNAi treatments result in the complete or near complete loss of MAPs and the disruption of the trough shape of the segment (dashed outlines in D and F). (G, H) Control females showing the state of proctiger concealment. (I) *Abd-B* RNAi results in the narrowing of the plate that conceals the proctiger, leaving it exposed. (J) Other female genital structures, such as the gonocoxae, are also affected. (K and L) *dsx* RNAi does not seem to alter the concealment of the proctiger or the genital segments.

L). The absence of any effects of *doublesex* knockdown in females suggests that this gene is not involved in the development of *G. odontogaster* female morphology during the final nymphal instar.

Discussion

We have demonstrated a tight negative correlation between a male sexually antagonistic trait (MAPs) and an apparent female counteradaptation (proctiger concealment) across several populations of *G. odontogaster*. These populations diverged recently from the North American species *G. buenoi* (Armisen et al., 2022; Damgaard et al., 2014), which does not carry these traits. Moreover, our phylogenetic reconstruction showed that when MAPs are lost, the ancestral state of the proctiger (exposed) is regained. Importantly, we provide experimental evidence for a genetic link between these two coevolving traits. The Hox gene *Abdominal-B*

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is involved in the development of both male and female sexually antagonistic traits, whereas the sex-determination gene *doublesex* is involved in the development of the male trait. Both genes are necessary for extending MAPs during development in males and their roles overlap significantly, whereas *Abdominal-B* is required for the concealment of the proctiger in the female. We first discuss the coevolutionary pattern of these two traits, and then their developmental biology and its implications for sexually antagonistic coevolution in this species.

Our analyses of the rapid coevolution of these sex-specific traits add to previous empirical examples of sexually antagonistic coevolution (Arnqvist & Rowe, 2002a; Bergsten & Miller, 2007; Dougherty et al., 2017; Koene & Schulenburg, 2005; Perry & Rowe, 2012; Rönn et al., 2007). Previous phylogenetic comparative analyses in the genus *Gerris* have revealed correlated evolution between sex-specific and sexually antagonistic genital and pregenital traits and showed that this is associated with the evolution of male–female interactions (Arnqvist & Rowe, 2002a, b). Our data mirror these findings at a microevolutionary scale, operating within a single species (see also Perry & Rowe, 2012), and further suggest that episodes of concerted escalation and de-escalation of sexual armaments can proceed rapidly in both sexes. This is the first clear example of de-escalation in both sexes that we are aware of. We suggest that de-escalation is an expected outcome of sexually antagonistic coevolution. Parker (1979) showed that sexually antagonistic coevolution may result in unresolvable evolutionary chases with escalation and de-escalation whenever male and female traits carry investment costs. In fact, models of sexually antagonistic coevolution that include natural selection on the antagonistic traits demonstrate that even where equilibria exist, changes in natural selection on both the traits and the outcome of male–female interactions (i.e., mating) will shift those equilibria, causing both escalation and de-escalation (e.g., Pennell et al., 2016; Rowe et al., 2005).

Natural selection on these coevolving traits is likely occurring in *G. odontogaster*. In terms of MAPs, these are known to be costly to males in terms of complicating the molt between the fifth larval instar and adulthood (Arnqvist, 1994). This should increase the risk of cannibalism and predation during molting, and the length of MAPs is negatively correlated with population density and predator presence across populations (Arnqvist, 1994). In terms of proctiger concealment in females, we suggest that this may also come at a cost. Since the proctiger primarily functions during egg deposition, trade-offs between efficacy as a resistance morphology versus egg deposition may render a more concealed proctiger suboptimal in terms of attaching eggs efficiently on aquatic substrates.

Ecological impacts on the economics of sexually antagonistic interactions are well-studied in water striders. Ecological factors, such as food abundance, predation, sex ratio, and population density, are all known to affect the costs and benefits of sexually antagonistic traits in water striders (Arnqvist, 1994; Arnqvist & Rowe, 2005; Perry & Rowe, 2018; Rowe, 1994; Rowe et al., 1994). For example, food manipulation experiments in *Gerris* have shown that hungry females become more resistant to mating (Rowe, 1992) and this biases mating success toward persistent males (Ortigosa & Rowe, 2002). In addition, high population density and male-biased sex ratios are both associated with relaxed sexual selection for long MAPs in *G. odontogaster* (Arnqvist, 1992a, b). Since *G. odontogaster* has a wide Palearctic distribution, the dynamic pattern of escalation and de-escalation of MAPs and proctiger concealment is consistent with variation in the ecological setting across its range. It is possible that the intensity of conflict varies across the wide geographic range of *G. odontogaster*, due to variation in the ecological setting of its populations. Future studies should examine whether and how the uncovered patterns of escalation and de-escalation of sexually antagonistic traits relate to variations in ecological factors.

Experimental manipulation of gene expression can provide information about the genetic programs shaping traits in both sexes, thus providing an important method to test whether correlated variation of male and female traits observed at the phenotypic level is linked to shared developmental genetic pathways. Depletion of two important developmental regulators, namely, *Abdominal-B* and *doublesex*, resulted in the loss or severe alteration of male and female traits. This result is consistent with similar findings in *Drosophila melanogaster* (Kopp et al., 2000) and in *Photuris* fireflies (Stansbury & Moczek, 2014). Our work shows that MAPs in *G. odontogaster* males are developmentally at least partially controlled by a network including these two proteins. In flies, *Abdominal-B* and *doublesex* interact to establish sexual dimorphism in abdominal pigmentation (Burtis, 2002; Kopp et al., 2000). *Abdominal-B* positively regulates the expression of *doublesex* and it is a one-way interaction (Wang & Yoder, 2012). Here, *doublesex* and another Hox protein (*sex comb reduced*) are known to regulate one another through a positive feedback regulatory loop (Tanaka et al., 2011). In our experiment, the roles of *Abdominal-B* and *doublesex* were similar, as knockdown of either gene in males resulted in the loss of MAPs and the alteration of the trough-shaped seventh abdominal tergite. This result indicates that the two genes interact and are part of a genetic network that shapes this male sexually antagonistic trait. The effects on two sex-specific male traits also show that these proteins have pleiotropic effects within males, which results in a genetic integration of MAPs and the shape of the seventh abdominal tergite. Perhaps most importantly, *Abdominal-B* RNAi also reduced the width of the dorsal plate leaving the proctiger exposed in females. This result demonstrates that this protein is required for the development of the coevolving male and female traits, a pleiotropic effect that should result in an intersexual genetic correlation between the traits, provided there is standing genetic variation in *Abdominal-B*. Several similar cases of other developmental regulators acting on male and female traits involved in reproduction have been previously reported. These include the role of the Hox gene *Sex combs-reduced* in the development of interacting but distinct male and female secondary sexual traits in the water strider *Rhagovelia antilleana* (Crumiere & Khila, 2019). In various beetles and milkweed bugs, distinct male and female genital traits are known to be shaped during development by overlapping genetic components (Aspiras et al., 2011; Macagno & Moczek, 2015), further supporting the conclusion that pleiotropy in the developmental genetic programs that shape sexual dimorphism in genital structures is widespread and may blur the distinction between intra- and interlocus sexual conflict.

Given that the male and female focal traits are different structures located on different segments, it would be easy to imagine them being underlain by independent loci. This is the common assumption of traits thought to be evolving independently and the use of interlocus coevolutionary models in efforts to understand coevolutionary diversification leans on this assumption (Arnqvist & Rowe, 2005). However, we have shown that *Abdominal-B* is necessary for the development of both of these traits. This result provides a rare empirical demonstration of a direct genetic link between intra- and interlocus sexual conflict (Mokkonen et al., 2016). If the observed evolutionary change in the two traits reflects either the evolution of *Abdominal-B* or its

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expression, then there is the potential for intralocus sexual conflict to play a role in their evolutionary dynamics despite the fact that these traits are not shared between the sexes. The theory has shown that a genetic correlation between male and female traits can act to destabilize sexually antagonistic coevolution, especially when traits are costly (Härdling & Bergsten, 2006; Härdling & Karlsson, 2009). This is particularly true when the genetic correlation is caused by pleiotropy, blurring the distinction between intra- and interlocus sexual conflict (Pennell et al., 2016), when cyclic dynamics involving de-escalation in both sexes can occur. Alternatively, the evolution of both of these traits may reflect the evolution in the downstream targets of *Abdominal-B*, and those targets may be independent of one another. A related example of this is the loss of abdominal pigmentation in *Drosophila santomea*, where both the evolution of *Abdominal-B* and its targets have played a role (Glassford et al., 2015; Liu et al., 2019). Our identification of *Abdominal-B* as a necessary component of the developmental network(s) underlying these rapidly coevolving traits enables more targeted studies of the networks underlying their evolution and their degree of overlap. This role of pleiotropy should be taken into account when interpreting patterns of phenotypic coevolution of the sexes involving distinct but interacting traits. Some fraction of loci underlying an adaptation in one sex might simultaneously code for related adaptations in the other sex, and this process will affect the dynamics of sexually antagonistic coevolution. A direct approach to test the extent of overlap between intra- and interlocus sexual conflict is to identify the genetic variation underlying the coevolving male and female traits. The *Gerris* study system offers this opportunity through the high within-species variation, which provides the possibility of mapping the genetic basis of male and female traits.

Supplementary material

Supplementary material is available online at *Evolution Letters*.

Data and code availability

All sequences used in this work are available in GenBank under the following accession numbers: PQ283678 and PQ283677. Raw data, measurements, and the R code for PGLS analysis are available in the supplementary online information.

Author contributions

A.K. and D.A. designed the research, A.K., D.A., C.P., and G.A. secured samples from various populations, D.A., C.P., P.R, and A.H. conducted the analyses, A.K. and C.P. wrote the initial version of the article, and L.R., G.A., A.H., and D.A. refined the final version of the article.

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Conflict of interest: Editorial processing of the manuscript was conducted independently of A.K., who is an Associate Editor of *Evolution Letters*. The other authors declare no conflict of interest.

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