The ecology of non-ecological diversification: how non-sexual selection affects within-environment diversification via sexual conflict

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ABSTRACT

Background: Sexual conflict is a pervasive force in nature that can instigate intersexual co-evolutionary arms races, potentially speeding processes of trait diversification and speciation, even in a constant environment.

Hypothesis: Sexual conflict interacts with ecology to influence the extent of sexual trait diversification among isolated populations. Specifically, trait diversification among ecologically similar populations is maximized under intermediate amounts of non-sexual selective pressure.

Features of model: I performed individual-based computer simulations of sexual conflict over mating rate to produce replicate populations that adapted to the same environment. I varied the strength of non-sexual natural selection, and measured the extent of within-environment diversification in sexual traits among populations.

Conclusions: Over short time scales (10,000 generations), sexual trait divergence among populations adapted to the same environment is maximized under intermediate amounts of selective pressure.

Keywords: mutation-order speciation, natural selection, sexually antagonistic co-evolution, sexual selection.

INTRODUCTION

How and why populations diverge and speciate remain fundamental questions in evolutionary biology (Coyne and Orr, 2004). While researchers have learned much about the genetic and environmental processes that underlie the origin of species since Darwin first referred to this 'mystery of mysteries' (1859), a great deal remains unclear as to how species multiply. Because the evolution of reproductive isolation revolves around changes in sexual behaviour and physiology, sexual traits and sexual selection have been of particular interest in seeking to understand divergence and isolation among populations (Panhuis *et al.*, 2001).

Among processes of sexual selection, sexual conflict is thought to be especially important in divergence, often acting as an 'engine of speciation' (Gavrilets and Waxman, 2002; Hayashi *et al.*, 2007; Bonduriansky, 2011). Sexual conflict occurs when the reproductive interests of males and females are not aligned with respect to a common trait, such as mating rate (Parker, 1979; Arnqvist and Rowe, 2005). Sexual conflict can promote sex-specific selection in which members of each sex seek to maximize their own reproductive output relative to same-sex competitors, even if reproductive gains come at a fitness cost to members of the other sex, which can lead to an ongoing evolutionary arms race between the sexes (Rice, 1996). Several verbal models have suggested that the particular sexually antagonistic co-evolutionary trajectories that populations proceed along are subject mainly to mutation, and isolated populations may therefore proceed down entirely different co-evolutionary pathways even in a constant environment (Holland and Rice, 1998; Andrés and Arnqvist, 2001; Arnqvist and Rowe, 2005). Sexual conflict is therefore considered a classic example of non-ecological, or mutation-order, speciation (Coyne and Orr, 2004, Schluter, 2009).

However, counter to its classification as a non-ecological driver of speciation, I hypothesize that sexual conflict interacts with ecological selection to dramatically influence patterns of sexual trait divergence among populations. Specifically, I predict that divergence via sexual conflict among populations adapting to the same environment (non-ecological divergence) will be maximized when non-sexual natural selection is of moderate strength.

Sexually antagonistic selection often leads to the exaggeration of traits used in displays to the opposite sex, weapons in intrasexual competitions and intersexual manipulations, and defences against such weapons (Andersson, 1994; Arnqvist and Rowe, 2005). However, these exaggerated sexual traits often come at a cost, such as through increased energetic investments (Andersson, 1994), increased harm from intrasexual competitors (Hansen and Rohwer, 1986), or increased risk of predation (Endler, 1980). If non-sexual natural selection (e.g. resource availability, predator density) influences the likelihood of mortality in response to sexual trait exaggeration, then certain environments will limit the extent of sexual trait exaggeration. Such limitations will inhibit trait diversification among isolated populations in these environments. That is, the nature of an environment will determine the extent of non-ecological diversification within that environment. In fact, Fricke *et al.* (2010) found such a pattern in experimental populations of seed beetles, where populations evolving under stabilizing selection showed more trait diversification than populations evolving under directional selection. However, counter to the conclusions of Fricke *et al.* (2010), I predict that within-environment sexual trait diversification will be maximized under intermediate levels of selective stress, rather than simply declining with the strength of natural selection.

The mechanism by which sexual conflict promotes rapid diversification in most models is via random, mutation-order processes (Holland and Rice, 1998; Andrés and Arnqvist, 2001; Coyne and Orr, 2004; Schluter, 2009). Specifically, the co-evolutionary trajectory along which populations proceed depends entirely on what sexually manipulative traits arise first. While males and females have been shown to evolve weapons and defences against their co-evolved counterparts (Arnqvist and Rowe, 2005; Perry and Rowe, 2012), there is no conceptual barrier to populations exploring multiple co-evolutionary trajectories simultaneously.

Populations evolving under strong pressure from non-sexual natural selection (a population with a high probability of pre-reproductive mortality) will resemble each other, as sexual trait exaggeration will be limited below what is possible through mutation. Therefore, each population will have similarly unexaggerated sexual traits. In contrast, in populations evolving under weak natural selection (low mortality probabilities), sexually antagonistic

evolution should lead to increasingly exaggerated manipulative and defensive sexual traits, limited only by the rate of mutations. As such, the gains of sexual trait exaggeration under sexual selection will outweigh the fitness losses from weak non-sexual natural selection, and sexual traits will diversify more than under strong natural selection. However, these populations under weak natural selection will potentially resemble each other in that they will continue to exaggerate their sexual traits as much as mutation will allow.

In order for mutation *order* to truly influence the evolutionary trajectories and outcomes of isolated populations, there must be a mechanism in place by which early mutations limit the influence of future mutations on a population's evolution. In the case of diversification via sexual conflict, an intermediate strength of natural selection can act as this mechanism. Intermediate natural selection will allow for the evolution of sexual trait exaggeration beyond what is possible under strong natural selection, and therefore allow for mutation to produce populations with distinct patterns of sexual trait exaggeration. However, the intermediate natural selection will limit exaggeration beyond a certain point, meaning that the exaggeration of one trait may inhibit the exaggeration of another once populations reach a threshold of sexual trait exaggeration. Therefore, an intermediate level of natural selection can ensure that the order of mutations limits the evolutionary outcome of sexual conflict and trait diversification (Fig. 1).

Sexual conflict has recently been seen to interact with ecology in various ways. For example, ecological context can determine whether sexually manipulative traits are harmful or advantageous to the other sex (Bondurianksy, 2014), influence the extent of trait divergence among replicate populations (Fricke *et al.*, 2010), and even promote the parallel evolution of male harm and female defence traits (Arbuthnott *et al.*, 2014). However, while these observations push our understanding of sexual conflict beyond the non-ecological context of previous landmark studies (e.g. Rice, 1996; Andrés and Arnqvist, 2001; Long *et al.*, 2006; Edward *et al.*, 2011), there is still a dearth of theory or evidence regarding how exactly the environment limits or directs the patterns of trait diversification via sexual conflict.

I explore my hypothesis using individual-based computer simulations. Numerical simulations allow me to test how particular environments with known effects on sexual and non-sexual traits affect trait diversification, and to vary the strength of selection acting on these traits. This gives me greater power for testing my hypothesis than empirical experiments would allow. Furthermore, these simulations extend previous models of sexual conflict by including multiple male and female sexual traits, rather than one, which allows me to explore sexual trait diversification. Using these simulations, I find support for my hypothesis. Under all parameters tested, sexual trait divergence among isolated populations adapting to the same environment is maximized under intermediate selective pressure. However, this result remains true only for a limited time, within the first 10,000 generations of population divergence, making this result important for processes of early divergence and speciation, but ephemeral in nature.

SIMULATION OVERVIEW

In these simulations, males and females interact, and females produce offspring. Sexual conflict is over mating rate: male fitness increases with increasing mating rate, while females have maximum fitness at an intermediate mating rate.

The probability that a male and female mate together is dependent on the interaction of five male and five female sexual traits. Specifically, this probability is affected by the
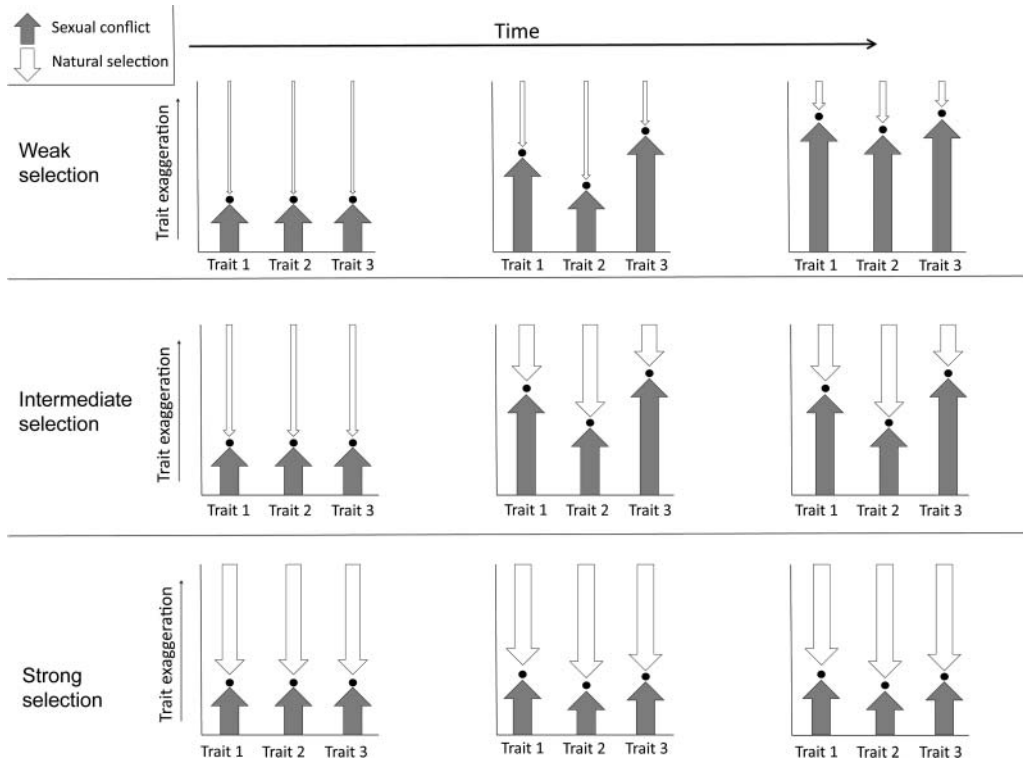
Fig. 1. Patterns of sexual trait exaggeration under different selection regimes. The solid dots represent the exaggeration of three theoretical sexual traits, influenced by sexual conflict (grey arrow) selecting for greater exaggeration, and natural selection (white arrow) selecting for less exaggeration. The thickness of each arrow represents its relative strength acting on sexual traits. As sexual traits become more exaggerated, the risk of mortality increases, and the strength of natural selection therefore increases. The strength of sexual conflict is kept constant in this figure for simplicity. Plots represent the patterns of sexual trait exaggeration through time (along the x-axis) under weak natural selection (top), intermediate natural selection (middle), and strong natural selection (bottom).

exaggeration of these traits, which reflects how much larger these traits are than what non-sexual natural selection alone would favour. Males increase mating probability with more exaggerated male display/manipulation traits, while females decrease this probability with more exaggerated female resistance traits. The number of times a female mates determines the number of offspring she produces, with the number maximized at an intermediate number of mates. Note that conflict is over the realized mating rates, which in turn are affected by mating probabilities in the simulations. So, while it is mating rate (within a narrow range of possible rates) that directly controls the conflict, mating rate and mating probability are intricately connected in the simulations.

Offspring inherit alleles from both parents coding for the size of each sexual trait. These offspring survive to reproductive age with a probability determined by their sexual trait exaggeration. The simulation tracks replicate populations for 20,000 generations in environments with varying strengths of non-sexual natural selection. Throughout the

simulated time span, I quantified the sexual trait diversity among replicate populations adapting to the same environment (how different populations are with respect to their sexual trait values). In quantifying sexual trait diversity, I determined the extent to which non-sexual natural selection influences diversification caused by sexual conflict.

COMPUTER SIMULATIONS

To test the effects of non-sexual natural selection on trait diversification through sexual conflict, I performed individual-based computer simulations adapted from the simulations performed by Arbuthnott *et al.* (2014). Like previous sexual conflict models (Gavrilets, 2000; Gavrilets and Waxman, 2002; Rowe and Day, 2006; Hayashi *et al.*, 2007), sexual conflict in these simulations is over mating rate, where male fitness increases with an increased number of matings, while females maximize fitness at an intermediate mating rate. The current simulations track changes in five male and five female sexual traits that influence mating rate as well as mortality under several selective environments.

I simulated diploid individuals that possess 10 autosomal loci coding for 10 traits (one diploid locus for each trait). These traits include five female defensive sexual traits (F_{1-5}) and five male sexual stimulation traits (M_{1-5}). While sexual traits are only expressed in the relevant sex, all individuals carry alleles for all traits. Each allele within each locus has a minimum value of 1 and no maximum. Each trait's value is the average of its two coding allele's values. Therefore, like the alleles coding for them, all traits have a minimum value of 1 and no maximum value.

Male stimulation traits increase the probability that females will mate during sexual interactions as the trait value increases. Such traits are often found in nature, and are the subject of much sexual selection research, such as increased visual displays, courtship vigour, and harassment behaviour. Female traits represent how susceptible females are to the stimulation traits of males, and the stimulation required to promote mating increases with increasing female defensive trait values. These traits can be directly defensive, such as morphologically resistant traits to male harassment and manipulations, or can simply reflect an increase in the required male stimulation (e.g. visual displays) to produce mating. Even though female mate choice based on male display traits is rarely thought of in terms of sexual conflict, male displays can take advantage of female preferences and induce them to mate beyond their optimum rate (Arnqvist and Rowe, 2005; Anrqvist, 2006). Therefore, both the male and female traits may be directly harmful to, or manipulative of, the other sex or not. However, the short-term antagonism of these traits does not affect my model, as it is only concerned with the overarching conflict over mating rate.

I generated a base population of 200 individuals with identical genotypes and a starting value of 1 for each trait. For each generation of simulations, each female interacted with five randomly selected males. For each interaction, mating occurred with a probability (Ψ) based on the female and male's sexual trait values:

$$\Psi = 1 - \exp\left(-\frac{\dfrac{M_1}{F_1} + \dfrac{M_2}{F_2} + \dfrac{M_3}{F_3} + \dfrac{M_4}{F_4} + \dfrac{M_5}{F_5}}{5}\right)$$

Overall, mating probability increases with increasing male stimulation values, and decreases with increasing female resistance values. Mating probability depends on the relative values

of the male and female sexual traits within the five male–female trait pairs (M_1 with F_1, M_2 with F_2, etc.). I paired these male stimulation and female resistance traits to some degree by dividing the male trait by its respective female trait (e.g. F_1 and M_1). Because these trait values are divided, the relationship between male and female traits within a trait pair influences mating probability to a greater extent than the relationship of sexual traits outside of these pairings (e.g. F_1 and M_2). Therefore, these five trait pairs can be thought of as potential male–female co-evolutionary pathways. For example, males with higher M_1 values (e.g. courtship rate) increase their probability of mating with any female. The most effective way for females to counteract this stimulation is to have a high resistance trait F_1 value (e.g. required courtship rate), although an increase in other sensitivity traits (e.g. required visual display, harassment resistance) will lower the probability of mating to a lesser degree.

After interacting with five males, females produce a number of offspring dependent on their mating rate (P, number of mates/5):

$$\text{offspring} = B_{\max} \exp(-s_{\text{sc}}(P - P_{\text{opt}})^2),$$

where $B_{\max}$ is the maximum number of offspring a female can produce (set at 10), P_{opt} is the optimal mating rate for females (set at 0.4, or 2 out of 5 males), and s_{sc} is the strength of sexual selection/conflict. The strength of sexual conflict affects how quickly offspring production decreases with departures from optimal female mating rate. For example, under weak sexual conflict ($s_{\text{sc}} = 5$), a female mated to four out of five males would produce four offspring; under intermediate sexual conflict ($s_{\text{sc}} = 8$), the same female would produce two offspring; and under strong sexual conflict ($s_{\text{sc}} = 12$), that female would produce just one offspring. The number of offspring produced by each female is rounded down to the nearest integer. Females who do not mate with any males produce no offspring.

For each offspring the female produces, a male with which the female in question mated is randomly selected as the father. This random assignment of paternity is a simplification of reproduction, as it ignores processes of post-mating female choice and sperm competition. However, males that are able to mate with multiple females will have a greater chance of siring multiple offspring than those males that mate with few or no females. Therefore, while a simplification, this system does select for males with higher mating probabilities via increased siring probabilities, which sets the stage for sexually antagonistic co-evolution. The genotype of the offspring is determined by randomly selecting one allele from each parent for each locus. Sex is then assigned with equal probability for each sex. Mutation also occurs at this time following a step-wise mutation model (Nei *et al.*, 1983), such that each allele increases or decreases in value by 1 (leading to a trait value change of 0.5) with a probability of μ (0.001 here). If any allele has the minimum value of 1, its value increases with probability $\mu/2$.

The offspring is then subject to natural selection, where its data are removed with probability

$$P(\text{mortality}) = s_n \sum (\mid F_x - F_{x_{\text{opt}}} \mid) \text{ for females}$$

and

$$P(\text{mortality}) = s_n \sum (\mid M_x - M_{x_{\text{opt}}} \mid) \text{ for males,}$$

where s_n is the selection coefficient. Therefore, the probability of mortality increases as a function of the deviation of each expressed trait from its natural selection optimum. The natural selection optimum for all sexual traits (i.e. F_x and M_x) was set at 1.

Reproduction and mortality processes are repeated for all females in a population, and the next generation is generated by randomly sampling 200 of the surviving offspring. The above process was repeated 20,000 times (generations) to produce a single selection line, and average male and female sexual trait values were recorded every 2000 generations. Ten replicate selection lines were generated for each environment tested. Each population was completely isolated, with no migration between replicates.

To explore how non-sexual natural selection influences sexual trait diversification within environments via sexual conflict, I altered the strength of natural selection, s_n. Therefore, these simulations were used to quantify the effect of non-sexual natural selection – which affects the probability of mortality based on sexual trait exaggeration – on sexual trait divergence via sexual conflict. s_n ranged from 0.0005 (very weak) to 0.02 (very strong). Under weak natural selection the costs of sexual trait exaggeration are minimal, whereas under strong natural selection sexual trait exaggeration is more likely to result in mortality before reproductive age.

To quantify sexual trait diversification within environments, I measured between-population distance in a multi-dimensional sexual trait space, where each dimension represents one of the 10 sexual traits, as in the framework used by Arnqvist (1998) and Fricke *et al.* (2010). To do this, I calculated the centroid (average) for each replicate line and its environmental centroid in this trait space (calculated from the 10 replicate lines within each environment). I then calculated the Euclidean distance between each population and its environmental centroid. Larger distances from the environmental average signify greater within-environment sexual trait divergence. I carried out these simulations and trait diversification calculations for three separate groups of selection lines, altering the strength of sexual conflict ($s_{sc} = 5$, 8, and 12). Each of these three groups was analysed separately, meaning that I was only concerned with the impact of the strength of natural selection, and not the strength of sexual conflict, on the extent of diversification within environments.

SEXUAL TRAIT DIVERSIFICATION IS MAXIMIZED UNDER WEAK TO INTERMEDIATE SELECTION

In all three simulation groups, the rate of sexual trait exaggeration through time decreased with the strength of non-sexual natural selection (Fig. 2a, b, c). For all environments, the optimal value for sexual traits to minimize mortality risk remained as the starting value of 1. Therefore, any exaggeration of these traits is entirely driven by the selective forces of sexual conflict, as exaggeration comes at a cost. The decrease of sexual trait exaggeration with increasing strength of natural selection indicates that, as expected, selective pressure on non-sexual traits can limit the extent to which sexual conflict can promote the exaggeration of sexual traits, as was also found in the models of Gavrilets (2000) and Rowe *et al.* (2003) for individual sexual traits.

Furthermore, the strength of non-sexual natural selection affected the extent of sexual trait diversification within environments under weak ($s_{sc} = 5$), intermediate ($s_{sc} = 8$), and strong ($s_{sc} = 12$) sexual conflict (Fig. 2d, e, f). On average, sexual trait diversification among populations under weak natural selection was greater than trait diversification under strong natural selection, confirming the empirical conclusions of Fricke *et al.* (2010). However, in all three simulation groups, the diversification index initially increased faster under intermediate levels of non-sexual natural selection, rather than being maximized in the environments with the weakest selection. The greatest diversification within the first 10,000 generations is
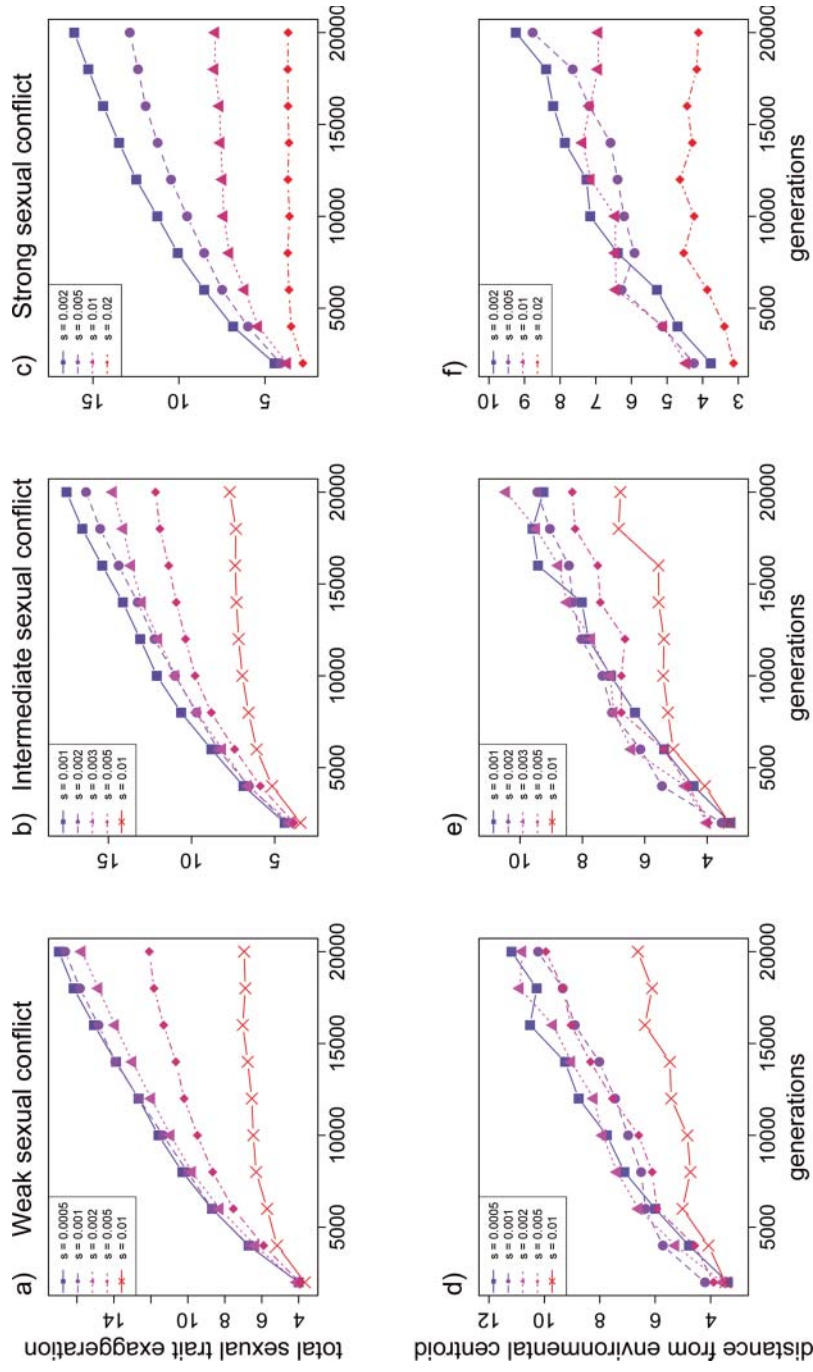
Fig. 2. Patterns of sexual trait exaggeration and diversification. (a–c) Mean total exaggeration of sexual traits (±SE) over 20,000 generations of simulated evolution among 10 replicate populations adapting to the same environment. Separate series represent different environments, which vary in strength of natural selection (s_n). Exaggeration represents the sum of deviation values from the natural selection optimum of all sexual traits (1) for each of 10 simulated populations within a common environment. (d–f) Mean within-environment sexual trait diversification (±SE) over 20,000 generations of simulated evolution among 10 replicate populations. Diversification was measured as the Euclidean distance of each simulated population from its environmental centroid. (a, d) weak sexual conflict ($s_{sc} = 5$), (b, e) intermediate sexual conflict ($s_{sc} = 8$), (c, f) strong sexual conflict ($s_{sc} = 12$).

seen when s_n is between 0.001 and 0.002 for $s_{sc} = 5$, when $s_n = 0.002$ for $s_{sc} = 8$, and when $s_n = 0.005$ for $s_{sc} = 12$. This matches my hypothesis that non-ecological sexual trait diversification is maximized under intermediate selection (Fig. 1).

While diversification within environments is maximized under intermediate levels of non-sexual natural selection within the first 10,000 generations, this pattern appears to vanish in generations 10,000–20,000. In all three simulation groups, diversification within the environment with the weakest selection catches up to that of their intermediate selection counterparts, at which time diversification within both environments becomes indistinguishable. It is likely that populations adapting to environments with intermediate levels of selection reach their natural selection limits with respect to sexual trait exaggeration within this time frame, which impedes further rates of trait diversification. This result implies that the difference in within-environment diversification is not permanent. However, this difference is present for the first ~10,000 generations of diversification, a time frame that is likely the most important with respect to the evolution of reproductive isolation and speciation via the forces of sexual conflict (Arnqvist *et al.*, 2000; Gavrilets, 2000; Gavrilets and Waxman, 2002; Coyne and Orr, 2004; Arnqvist and Rowe, 2005), making differences on this time scale biologically important.

These simulations have a number of limitations, such as the assumption that separate traits and loci act independently. One of the most obvious ways that evolutionary changes can limit future evolutionary pathways is through pleiotropy, where genetics or selection makes certain genetic or phenotypic combinations untenable (Rice and Hostert, 1993; Hawthorne and Via, 2001; Rundle and Nosil, 2005). While my simulations cannot encompass such processes, I argue that evolutionary pathways are not always straight, and experiencing co-evolution of one sexually antagonistic pathway does not bar populations from progressing along one or more alternate antagonistic pathways. Verbal models of diversification via sexual conflict too often assume that a step along one evolutionary pathway is a step away from another (e.g. Holland and Rice, 1998; Andrés and Arnqvist, 2001), but co-evolution is unlikely to be so linear or one-dimensional.

CONCLUSIONS

I used individual-based computer simulations to assess how the nature of selection affects the extent of within-environment (i.e. non-ecological) sexual trait exaggeration and diversification. As expected, strong non-sexual natural selection limited the extent of trait exaggeration through sexual conflict. However, weak natural selection did not maximize trait diversification. Multiple simulations show a pattern of an initial increase in the rate of diversification under intermediate levels of selection (Fig. 2). At intermediate levels of selection, mutations that exaggerate sexual traits early during adaptation can limit the extent of exaggeration later in the process, while still allowing for greater exaggeration than under strong selection (Fig. 1). These results suggest that intermediate selection strength facilitates processes integral to mutation-order speciation (Schluter, 2009).

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