Ecology and Evolution of Mating Behavior in Freshwater Amphipods

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Abstract and Keywords

Freshwater amphipods are used as a research model for studies of female mating preferences, the nature of benefits that drive female choice, and the influence of sexual conflict on female preference. Direct selection on female mating preferences is hypothesized to occur during the contact pairing phase, because costs incurred or benefits gained by females during pairing are likely to depend on traits of guarding males. Comparative studies of ecomorphs in the genus *Hyalella* shed light on the evolution of female preference and preferred male traits. In species from habitats where large body size is favored by ecological processes females prefer larger males. In species subject to intense fish predation, however, mortality selection favors small body size and female preference for larger males is weak. These divergent mating biases are consistent with direct and indirect selection on female preference acting under the disparate regimes of natural selection faced by the ecomorphs.
Freshwater amphipods have been models for the study of male time-investment strategies and sexual conflict over mate-guarding duration (reviewed in Jormalainen 1998). Other features of their mating biology, most notably, sexual selection and the mechanisms mediating it, have received comparatively little attention despite the important roles these are likely to play in evolution of mating behavior, reproductive traits, and sexual dimorphism (Andersson 1994). We focus on these areas by assessing the state of current knowledge of sexual selection and related issues, discussing the costs and benefits that are likely to drive sexual selection, and describing comparative studies of interspecific ecomorphs in *Hyalella* that hold promise for shedding light on these issues. We conclude with a discussion of the prospects and challenges that freshwater amphipods offer as empirical models to address current conceptual problems in the evolution of mating behavior.

Our treatment is necessarily selective and often speculative and is offered with the hope that it will stimulate discussion and, better yet, empirical studies that fill the many gaps in our understanding of sexual selection in this group. To a large degree, the current issues that confront advances in our knowledge of mating biology of amphipods, such as the relative roles of sexual conflict and mate preference and the maintenance of genetic variation for sexually selected traits, are precisely the challenges faced by the broader discipline of sexual selection. Thus, empirical advances in amphipod mating behavior have the potential to clarify fundamental issues in behavior and evolution.
Overview of Reproductive Biology and Mating System

Some key features of the reproductive biology of freshwater amphipods are important for understanding evolution of their mating system and behavior. As in many other crustaceans, the opportunity to fertilize eggs is limited to the period just after the female's molt (Sutcliffe 1992). This time-limited opportunity for fertilization, together with external fertilization, favors the evolution of contact mate guarding, a ubiquitous behavior in freshwater amphipods (Ridley 1983). In precopulatory mate guarding (referred to here as “pairing” for simplicity), males are positioned dorsal to the female and grasp the female's integument with prehensile thoracic appendages called gnathopods. Males hold females in this way until the female molts, at which time ova are passed into the female's external brood chamber, where they are fertilized by the guarding male (Sutcliffe 1992). Males discontinue pairing soon after fertilization, and females do not store sperm. Females carry the developing embryos until the embryos hatch and leave the brood chamber, before or coincident with the next female molt. Females are iteroparous and generally capable of producing a new clutch with each molt. Timing of female receptivity is usually asynchronous among females.

Pairs do not remain together after fertilization, and sexes are promiscuous. Shuster and Wade (2003) classify this typical amphipod mating system as coercive polygynandry because male coercion can involve costs to females. As we discuss below, it is very difficult to distinguish between successful male coercion and female selectivity (Cordero and Eberhard 2003), and it is not clear that entirely coerced pairings ever occur in freshwater amphipods. Nonetheless, sexual conflict over precopulatory pairing is likely (see chapter 8).

(p.149) Sexual Selection
Mating is characterized by intrasexual competition and by complex intersexual interactions reflecting a tension between cooperation and conflict (Trivers 1972). Evolutionary dynamics that drive sexual selection and shape mating behavior derive from multiple mechanistic processes, including sexual conflict, intrasexual competition for access to mates, direct and indirect selection on female preference, and sensory bias. Much effort, both theoretical and empirical, has focused on assessing the relative prevalence of these mechanisms and the circumstances under which they might operate (Andersson 1994, Kokko et al. 2002, Chapman et al. 2003). Most recently, there has been an emerging recognition that these processes are likely to operate simultaneously and interactively in the evolution of mating traits (Kokko et al. 2003, Arnqvist and Rowe 2005, Cordero and Eberhard 2005, Kokko 2005), suggesting that synthetic research approaches are needed. In amphipods, few studies have considered the nature and scope of sexual selection and its ultimate consequences for traits associated with intrasexual and intersexual mating interactions. In this section, we explore components of the amphipod mating sequence, focusing on the roles of each sex, and the variety of ways that sexual selection may shape mating traits in both sexes.

Searching Phase

In freshwater amphipods, time-limited and asynchronous female receptivity give rise to male-biased operational sex ratios (Jormalainen 1998), a condition expected to promote intense competition among males for mating opportunities (Emlen and Oring 1977, Shuster and Wade 2003). One way males may enhance mating success is to increase investment in mate searching behavior, as is common in amphipods (Ridley 1983). Searching may be costly, however, exposing males to higher rates of predation and parasitism. Indirect evidence for these costs comes from adult sex ratios in the field, which are often female biased (Ward 1986, Wellborn and Bartholf 2005), and higher parasite loads in males, which carry an associated reduction in mating success (Ward 1986, Bollache et al. 2001). Searching also may be energetically costly (Sparkes et al. 2002). For example, unpaired *Gammarus pulex* males had lower energy reserves than did paired males in a natural population (Plaistow et al. 2003), and because males had the potential to replenish energy reserves while
paired, costs of mate searching behavior may have contributed to low energy reserves in unpaired males.

Mate searching costs in males can influence the evolution of pairing duration (Jormalainen et al. 1994, Yamamura and Jormalainen 1996, Cothran 2004; see also chapter 8). Predation risk (Cothran 2004) and feeding rate (Robinson and Doyle 1985), for example, may differ between searching and pairing phases, altering optimal pairing duration (Yamamura and Jormalainen 1996). Search costs can also play an important role in determining the intensity of intersexual conflict over guarding duration (Parker 1979, Jormalainen 1998). Resolution of guarding conflict should depend on the relative ability of each sex to influence the outcome of the conflict and on each sex’s fitness payoff from alternative outcomes (Yamamura and Jormalainen 1996). When male search costs are high, males and females may differ in the relative costs of pairing because, for males, the cost of pairing may be offset by the cessation of (p.150) searching costs, but because females do not suffer a search cost, any cost of pairing represents a net cost (Jormalainen 1998, Cothran 2004). Such an asymmetry between sexes sets the stage for intersexual conflict over guarding duration (see chapter 8), and traits that are important in determining the outcome of this interaction may be especially prone to evolutionary change as a result of such conflicting interests.

Costs of mate searching will depend on ecological conditions (Andersson 1994, Rowe 1994; see also chapter 10). For example, freshwater habitats vary in the form and intensity of predation, which may lead to variation in the direction and intensity of selection acting on male activity (Strong 1973, Wellborn et al. 1996). Other aspects of an individual's habitat, including resource availability, temperature, sex ratio, and the temporal and spatial distribution of females, are likely to affect searching costs (Jormalainen 1998) and thus foster interpopulation and interspecific variation in mating behavior (Strong 1973, Wellborn 1995, Jormalainen et al. 2000, Wellborn and Bartholf 2005).
Assessment, Resistance, and Pairing

Encounters between males and females may precipitate pair formation, but often they do not (Elwood et al. 1987, Sparkes et al. 2000, Strong 1973). Qualities of individuals that determine whether pairing will occur have been considered primarily from a male perspective. Female characteristics that enhance their value to males include larger size, owing to its correlation with fecundity (Ward 1988, Wellborn 1995), and shorter time until the female molt, which decreases missed opportunity costs for guarding males and lowers costs arising from reduced feeding or increased predation during pairing. Additionally, small female size, relative to male size, may be favored under some conditions due to reduced energetic costs of carrying females (Adams and Greenwood 1987). Traits of males that influence their value to females have received much less attention but are likely to be important considering the growing recognition of the role female behavior plays in mediating pair formation (Borowsky and Borowsky 1987, Sutcliffe 1992, Jormalainen 1998, Sparkes et al. 2000, 2002).

In this section, we explore the potential for females to exercise choice in pairing and examine how sexual selection driven by female preference may operate in freshwater amphipods.

The degree of female control in mating is unclear. Most researchers interpret pair formation as being under mutual control of both sexes, although not necessarily with both sexes having equal influence (see Table 2 in Jormalainen 1998). Some researchers interpret pair formation to be controlled strictly by male choice, with females being passive (Jormalainen 1998). These conflicting interpretations probably reflect some degree of behavioral differences among species studied, but female passivity and female control have sometimes been reported in the same species (Birkhead and Clarkson 1980, Ward 1984, Elwood et al. 1987). We use the term “female control” to indicate circumstances in which females have the ability to prevent some or all unwanted pairings, and “female preference” to refer to circumstances in which female traits (resistance behavior, chemical signals, etc.) cause some male phenotypes to have higher mating success than others. Our intuition is that female control and preference are likely to be common in amphipods both because observations of female resistance are common, and because costs and benefits of when and with whom to pair are
likely (p.151) to differ between sexes (Jormalainen 1998, Arnvist and Rowe 2005), and thus there should be selection on both sexes’ ability to control pair formation.

Female physical “resistance” to male mating attempts is common in animals (Parker 1979, Crudgington and Siva-Jothy 2000, Hosken et al. 2003, Arnvist and Rowe 2005), including amphipods and isopods (Jormalainen 1998, Sparkes et al. 2000). The functional role of female resistance is often attributed to minimizing naturally selected costs of pairing (Jormalainen 1998, Chapman et al. 2003). For example, female amphipods may use resistance behavior to counter the costs of prolonged precopulatory pairing with males (Jormalainen and Merilaita 1995; see also chapter 8). Another, but not mutually exclusive, explanation for resistance behavior is that females exercise active mate preference through selective resistance (Sparkes et al. 2002, Cordero and Eberhard 2003, Kokko 2005). In either case, resistance behavior may cause male mating success to vary across male phenotypes, imposing sexual selection on male traits.

The issue of the extent to which each sex is able to exercise control of mating is important in understanding the evolution of mating behavior (Fincke 1997) but is exceedingly difficult to evaluate in amphipods due in part to the nature of the tactile interaction that occurs at encounter. Is what appears to be “resistance behavior” a general female rejection of all males, or is it sometimes assessment behavior that facilitates female preference? Is resistance an honest signal to males indicating that the female is far from her molt, and thus is to the mutual benefit of both individuals to forgo pairing, or is resistance a sometimes deceptive signal used for female preference?

Although studies that manipulate female behavior are valuable (Jormalainen and Merilaita 1995), such studies could be misleading if males use female behavior to assess receptivity or other qualities of females. Ultimately, studies that focus on fitness consequences accruing to females and males exposed to different choice regimes may be particularly informative in elucidating the role of each sex in pairing dynamics (Cordero and Eberhard 2005).

A large male mating advantage is common in freshwater amphipods (Birkhead and Clarkson 1980, Ward 1984, Wellborn 1995, Jormalainen 1998), and variance in other male traits, including gnathopod size and antennal length, may influence
pairing success (Conlan 1991, Wellborn 1995, 2000) but have received less attention. Most explanations for a large male mating bias have centered on competition among males for access to mates, with larger males having an advantage in scramble competition or takeover attempts (Ward 1988, Bollache and Cezilly 2004). Although intrasexual competition for receptive females should be intense in freshwater amphipods, direct takeovers of paired females by single males are apparently rare (Strong 1973, Elwood et al. 1987). Scramble competition among males is likely, however, and should select for high rates of searching activity (Ridley 1983). Higher activity will increase encounter rates with females, but its effectiveness in securing mates will also depend on female preference, provided that females can exercise control in pairing (Sparkes et al. 2000). For example, if females control pair formation and discriminate among males based on body size, then we must look to female preference as an important determinant of size-biased pairing in males.

Evolution of mating preference requires that selective mating yield higher fitness than indiscriminate mating (Andersson 1994, Kokko et al. 2003). Variation in mate quality can cause direct selection on female mating biases when nonrandom mating (p.152) increases female fecundity or viability (Price et al. 1993, Kokko et al. 2003). In amphipods, direct selection on female mating preferences may be common during the precopulatory pairing phase, but we are not aware of studies explicitly evaluating this issue. Precopulatory pairing might influence predation risk (Ward 1986, Cothran 2004), foraging (Robinson and Doyle 1985, Sparkes et al. 1996), habitat use (Sparkes et al. 1996), and mobility (Adams and Greenwood 1987), and it seems likely that the impact of these effects on a female will often depend on her mate's body size or other traits.

A second potential mechanism for the evolution of female preference in amphipods is indirect selection that occurs when mating preferences increase offspring fitness (Kokko 2001). Females gain indirect fitness benefits because preferred male traits are genetically correlated with components of fitness, and offspring inherit these “good” genes. The potential importance of “good genes” indirect selection in driving female mating preference has been debated (Andersson 1994, Gavrilets et al. 2001, Cameron et al. 2003, Cordero and Eberhard 2003), with much attention focused on how genetic
variance in male fitness is maintained, and the related issue of whether indirect selection can be sufficiently strong to drive mating preferences when preference is costly (Kokko et al. 2003). Recent studies have at least partially clarified this issue by demonstrating that additive genetic variance in fitness related traits can be considerable (Burt 1995, Pomiankowski and Møller 1995, Houle 1998).

Explanations for how genetic variation in fitness is maintained have focused on the idea that male traits that are targets of selection by female preference are condition-dependent traits that capture genetic variation at the level of the genome (Houle and Kondrashov 2002, Tomkins et al. 2004, Hunt et al. 2004). Traits that are costly to produce, such as body size, may be genetically correlated with condition, or overall quality, of an individual because large size is the product of all of the developmental, physiological, and behavioral processes that underlie size. Because such condition-dependent traits are determined by the collective influence of a large number of genes, the traits are large targets for mutation, allowing substantial genetic variance to be maintained (Rowe and Houle 1996, Houle 1998). Some empirical studies are now beginning to illuminate the issue of indirect selection on female choice (Jones et al. 1998, Iyengar and Eisner 1999, Hine et al. 2002), including a study of crickets demonstrating that female preference for attractive males can provide indirect fitness benefits to females that outweigh direct costs (Head et al. 2005). In an isopod, Sparkes et al. (2002) manipulated male condition by causing some males to expend glycogen reserves and found that males with higher condition had greater pairing success because females resisted more vigorously in encounters with low-condition males.

Male traits for which preference is most likely to evolve are those that reliably indicate fitness, and thus preferred traits should be those having fitness correlations that are relatively unaffected by environmental variance and genotype by environment interactions (Hunt et al. 2004). Body size or gnathopod size, for example, might satisfy this requirement because, although these can vary with resource levels or other environmental conditions, relatively large size might always denote high genetic quality in habitats where larger size is beneficial. That body size and correlated traits may be targets of sexual selection is particularly interesting because amphipods have indeterminate growth, implying that
phenotypic variance in size results from variance in both growth rate and age. Provided that some mortality occurs across mature (p.153) age classes, variation in size due to age differences will also reflect variation in viability. Thus, size-biased mating can impose sexual selection on growth and viability (Trivers 1972, Kokko 1998), presumably mediated through the underlying traits, such as resource acquisition ability, that cause variation in growth and viability (Tomkins et al. 2004). Furthermore, both growth rate and viability are likely to be condition dependent, suggesting a mechanism for maintenance of additive genetic variance in these traits (Houle 1998). As a methodological note, indeterminate growth in amphipods greatly hinders quantification of sexual selection intensity directly from mating biases because mating biases do not necessarily denote variation in lifetime mating success (Arnold and Wade 1984). Nonetheless, mating biases with respect to size imply sexual selection can act to the extent that size variance is due to differential growth and survival (Wellborn 1995).

Ecological Context and Sexual Selection in *Hyalella* Ecomorphs

The causes and phenotypic outcome of sexual selection are mediated by its interaction with natural selection (Partridge and Endler 1987, Andersson 1994, Kokko et al. 2003; see also chapter 10). Because natural selection is driven largely by ecological processes, ecological context may modulate the evolution of mating behavior in important ways (Houde 1997, Shuster and Wade 2003). In this section, we describe comparative studies of interspecific ecomorphs within a group of *Hyalella* amphipods (Fig. 7.1). The ecomorph species experience disparate ecological conditions that determine the benefits and costs of mating activities, which in turn shape evolution.
(p.154) of mating behavior. Thus, understanding the role of ecological factors in generating interspecific diversity in mating behavior can illuminate the mechanisms by which sexual and natural selection interact to form mating phenotypes.

Figure 7.1 Females of large (top) and small (bottom) ecomorphs within the *Hyalella azteca* species complex collected in Oklahoma. These species are undescribed (Wellborn et al. 2005).

Figure 7.2 Mean (±1 SD) body size and maturation size (diamonds) of large and small regional ecomorphs in the *Hyalella azteca* species complex. Notation indicates collection site (OK, Oklahoma; MI, Michigan; OR, Oregon). Letter designations for Michigan small ecomorphs indicate separate species.
Hyalella amphipods are common in permanent freshwater habitats throughout much of the New World. Here, we explore diversity in mating behavior among “interspecific ecomorphs” (i.e., sets of species that differ consistently both in habitat and in adaptive traits that mediate ecological success in the different habitats). These ecomorphs differ in body size and life history (Fig. 7.2) and occur in at least three regions of North America: Oregon (Strong 1972, 1973), Michigan (Wellborn 1994), and Oklahoma (Wellborn et al. 2005). The ecomorphs are undescribed species that fall within a broader array of previously hidden species diversity recently discovered through genetic analyses (Witt and Hebert 2000, Wellborn et al. 2005). Ecomorphs within the same geographic region segregate among local habitats based on the habitats’ ecological qualities. “Small ecomorph” species mature early, maintain a small adult body size, and occur in habitats where they are subject to intense predation by fish, particularly sunfish in the genus Lepomis. “Large ecomorph” species mature at a large body size and occur in habitats lacking this intense predation by fish but are subject to predatory invertebrates and some fish predation (Wellborn et al. 2005).

Ecomorph phenotypes are adaptive, given the disparate ecological conditions of their habitats (Wellborn 1994). The ecology of regional ecomorphs is best understood for Michigan species, but similar mechanisms are likely to operate in the other regions (Strong 1973, Wellborn et al. 2005). In Michigan, small ecomorph species experience (p.155)
intense size-selective predation by *Lepomis* sunfish, and mortality increases with body size (Fig. 7.3). In contrast, the large ecomorph species is found in fishless habitats where larval dragonflies are the primary predators. These predators impose heaviest mortality on smaller, juvenile individuals, and mortality declines with increasing body size. Ecomorph differences in maturation size and adult body size are consistent with life history adaptation under these disparate mortality regimes (Taylor and Gabriel 1992).

*Figure 7.3* Mean (±1 SD) daily mortality in natural habitats for large and small ecomorphs in Michigan replicated in two years. Based on data from Wellborn (1994).
Hyalella Mating Behavior

Our observations of mating behavior in Michigan and Oklahoma Hyalella species (Wellborn 1995, Wellborn and Bartholf 2005) largely coincide with those of Oregon ecomorphs reported in Strong (1973). Males actively search for females. Movement of males appears haphazard, and there is no evidence of waterborne chemical communication between the sexes (Strong 1973, Wellborn and Cothran, unpublished observations). Because females do not appear to search, males bear costs of mate searching, which may include elevated predation risk. Indeed, female-biased sex ratios are typical for Hyalella. For example, large- and small-ecomorph populations in Oklahoma were 20% and 34% male, respectively (Wellborn and Bartholf 2005), and Michigan ecomorph populations were approximately 40% male (Wellborn 1995). The operational sex ratio, however, is male biased, because only about 9% of females are expected to become receptive each day, assuming an 11-day molt cycle that is asynchronous among females (Wellborn 1995, Othman and Pascoe 2001), whereas virtually all unpaired males are able to pair. Operational sex ratios for Oklahoma ecomorph species were approximately 72% and 84% male for large and small species, respectively, and 86% male in Michigan ecomorph species (calculated from Wellborn 1995, Wellborn and Bartholf 2005).

Females, through resistance behavior, appear to control pair formation in Hyalella (Strong 1973, Wellborn 1995). While actively searching, males appear to attempt to pair with each female encountered, and females often appear to resist. Resistance behavior in female Hyalella is similar to that reported in other amphipods and isopods (Ward 1984, Hunte et al. 1985, Borowsky and Borowsky 1987, Elwood et al. 1987, Caine 1991, Jormalainen and Merilaita 1993, Sparkes et al. 2000) and includes thrashing and tightly curling their body (Strong 1973, Wellborn 1995). Because it is associated with failed pairing attempts, we interpret this behavior to indicate that females can control the onset of pairing (Wellborn 1995), a conclusion shared by Strong (1973). This inference is also supported by ongoing experimental work showing that males pair earlier in the female molt cycle in trials with inactive females, for which resistance behavior is experimentally removed by anesthetization, than with active females (R.D. Cothran, unpublished observations). A detailed study of a
freshwater isopod recorded similar behavioral interactions and concluded that females can control pairing by persisting in resistance behaviors (Sparkes et al. 2000).

Timing of the onset of precopulatory pairing is likely to involve intersexual conflict and will depend on ecological conditions that shape costs of pairing (Härdling et al. 2001; see also chapter 8). Pairing is beneficial for both sexes very near the female's molt, but net benefits decline progressively as the time to the female's molt increases because costs of pairing accrue with pairing duration (Jormalainen 1998). Sex differences in costs of pairing generate disparity between sexes in the optimal duration of pairing. How is intersexual conflict in pairing duration resolved in *Hyalella*? Although manipulative experiments are needed, we suggest that current evidence indicates conflict may be largely resolved in favor of females. That males appear indiscriminant in attempting to pair with females, whereas females successfully resist males, offers observational evidence consistent with our interpretation. Additionally, the observational evidence is corroborated by the recently completed study described above, showing that males guard anesthetized females longer than they do active females. These results suggest that optimum guarding duration is greater for males than for females, as may be typical in amphipods (Jormalainen 1998).

Although males may suffer a missed opportunity cost by pairing with a female that is too far from her molt, receptive females (i.e., females that ultimately accept males) may always be sufficiently near their molt that they fall within the male optimal pairing duration. If true, female receptivity acts as a reliable indicator of female quality for males. One difficulty with this interpretation, however, is that males could profit from forced pairings with females whose molt stage falls within the male optimum guarding duration, but not yet within the female optimum. Because of the nature of the tactile interaction that precedes pairing, it is very difficult to differentiate male force from female preference enacted by selective resistance (Cordero and Eberhard 2003). If forced pairing does occur, this behavior raises the question of how males assess female molt stage.

Costs of forced pairing for males may be high, especially if males cannot determine the time remaining until a female molts and thus cannot assess the intensity of missed
opportunity costs (Jormalainen 1998). Given that information about the female’s internal physiological state “belongs” to females, it is not clear why females would reveal this information to males when its use precipitates a fitness cost to females. One possibility is that females cannot control information about their molt stage, for example, if molting hormones unavoidably leak from females. Indeed, in some amphipod species, waterborne or contact chemicals released by females influence pairing (Lyes 1979, Borowsky and Borowsky 1987, Borowsky 1991, Sparkes et al. 2000), but it is not clear whether females control display of chemical cues in these species, and it is not clear whether females suffer a direct fitness cost as a result of the cues, as would be expected if males use cues in coercive pairings. An alternative possibility for male assessment of female molt stage is that males judge the time to female molt based on intensity of female resistance (Jormalainen 1998), but females may be able to deceive males by altering resistance. We know of no a priori reason why females must honestly signal their time to molt during resistance behaviors. Clearly, better empirical understanding of the dynamics of coercive pairing and information transfer is needed to resolve these issues.

Ecological factors may shape pairing duration by influencing the costs of guarding, and to the extent that these ecologically mediated costs differ between sexes, they may shift levels of intersexual conflict (Cothran 2004; see also chapter 8). In *Hyalella*, increased susceptibility to predation is a potentially important cost of pairing, and because predation regime differs between habitat types, the two *Hyalella* ecomorphs are likely to be differentially affected by predation. Strong (1973), studying ecomorphs in Oregon, found that mean pairing duration in the large ecomorph species was 4.8 days, whereas the small species pairs for only 2.6 days. In Oklahoma, large and small ecomorph species pair for an average of 3.0 and 1.4 days, respectively (Wellborn and Bartholf 2005). Strong (1973) hypothesized that size-selective predation by *Lepomis* and other fish in the small species’ habitat causes elevated predation risk for paired individuals, and this risk drives the evolution of short pairing duration.

This hypothesis was tested experimentally in Oklahoma ecomorph species (Cothran 2004). In fish predation treatments, pairs of the small species were consumed about twice as frequently as unpaired individuals, suggesting a
substantial cost of pairing in fish habitats. Although fish always consumed both individuals in pairs, the cost of pairing was greater for females because mortality of single males in the experiment tended to be higher than that of single females, perhaps because male searching behavior makes them more conspicuous to fish. In large ecomorph habitats, predatory invertebrates, especially dragonfly larvae, are common. These predators had an effect opposite to that of fish, consuming single individuals more frequently than paired individuals. Thus, at least in terms of predation risk, pairing may be beneficial for large ecomorph individuals, and this benefit was similar between sexes. Overall, the experiment points to habitat differences in predation as a likely cause of the ecomorph differences in pairing duration.

A further implication of the study by Cothran (2004) is that small-species females, given their high cost of pairing, may be especially likely to delay pairing until very close to their molt and that intersexual conflict over pairing duration may be high. Furthermore, by delaying pairing until she is very near her molt, a small-species female obtains the added benefit of reduced risk to her developing offspring because offspring often leave the brood pouch a day or two before the female's molt. In a study of Oklahoma ecomorphs, few small-species females in pairs carried embryos or offspring in the brood pouch, compared with single females and with paired females of the large species (Fig. 7.4). Thus, elevated predation risk may drive short pairing duration in the small species due to the twofold benefit of increased survival for the female and increased survival of her current offspring.

(p.158)
Mating Biases and Sexual Selection

Sexual selection is influenced by natural selection in multiple ways. Expression of traits subject to directional sexual selection is balanced by countervailing natural selection (Kokko et al. 2002). Form or intensity of direct benefits that shape evolution of mate choice may depend on ecological conditions (Andersson 1994). Also, mate preferences driven by indirect benefits can arise because sexual selection favors females that mate with males possessing traits favored by natural selection (Kokko et al. 2002), and thus it follows that preferences will depend on the regime of natural selection operating in a system.

Variation among populations or species in the action of natural selection, therefore, can produce variation in the action or outcome of sexual selection (Partridge and Endler 1987, Houde 1997). In this section, we explore how differences in natural selection may interact with sexual selection to give rise to mating biases observed in *Hyalella* ecomorphs. A full understanding of selection mechanisms operating in this system will require extensive experimental work, some of which is underway. At present, our approach is necessarily limited to documenting ecomorph differences in mating biases.

![Figure 7.4](image.png)

*Figure 7.4* Percentage of unpaired (dark bars) and paired (light bars) females with embryos in the marsupium for the small and large *Hyalella* species in Oklahoma. Because the small ecomorph species delays pairing until late in its molt cycle, most females have released offspring before pairing begins. Based on unpublished data collected for Wellborn and Bartholf (2005).
and attempting to make sense of these differences in the context of the ecomorphs’ disparate ecological contexts.

Mating success of male *Hyalella* is often dependent on male body size (Wellborn 1995, Wellborn and Bartholf 2005). Although other traits may also be under sexual selection (Wellborn 1995, 2000), we focus here on body size because we understand much about how size affects ecological success of these species (Wellborn 1994, 2002) and thus how ecological factors may influence sexual selection. In Oklahoma ecomorphs, field studies demonstrated a large male mating advantage for both ecomorphs, but ecomorphs appeared to differ in the form of the relationship between body size and pairing success (Wellborn and Bartholf 2005). In the large ecomorph, (p.159)

Pairing success increased continuously across male size. Pairing success in the small ecomorph, however, exhibited a threshold relationship in which smaller males had low pairing success, but pairing success did not differ across intermediate- to large-sized males. This field pattern was also evident in a mate choice experiment showing that, in the large ecomorph species, a female tended to mate with the larger male regardless of the absolute size of the males in the trial (Fig. 7.5). Size-biased pairing in the small species, however, depended significantly on the absolute size of the smaller male in the trial. Males of the smallest mature size class had low pairing success, but above this smallest size class, larger male size did not enhance pairing success. This pattern of mating biases is also seen in Michigan ecomorphs (Wellborn 1995), suggesting that similar ecological processes cause similar mating behavior.
Mating biases suggest that sexual selection in large ecomorphs pushes males toward ever larger size. In contrast, sexual selection in small ecomorphs imposes only a minimum threshold size at the lower extent of the adult size range, pushing male size over the threshold, but not larger. The disparate forms of these mating biases have an obvious correspondence to the disparate ecological value of body size for the two ecomorphs. Females prefer larger males in environments where larger size equates to greater ecological success (Wellborn 1994, 2002), but in environments where large size entails a mortality cost, females do not prefer larger males above a threshold size. Our challenge is to understand the mechanisms of sexual selection operating in *Hyalella* ecomorphs and how these drive observed mating biases.

*(p.160)* Our understanding of the *Hyalella* mating system points to two possible forms of intrasexual selection among males. First, direct male–male interactions might occur if a single male is able to displace a guarding male. Such takeovers, however, appear to be rare in *Hyalella* and unlikely to play a significant role in sexual selection (Wen 1993). For example, Strong (1973) reported that takeovers did not occur in laboratory trials, even when paired males were smaller than unpaired males. A second mechanism of intrasexual selection in *Hyalella* is scramble mate competition, in which males attempt to find and guard receptive females before females are guarded by competing males. This mechanism is made more likely by the rarity of takeovers because males must find...
receptive, but unguarded, females. Sexual selection arising from scramble mate competition favors male traits, such as high activity rate, that increase encounters with receptive females. Although male *Hyalella* actively search for mates, currently there is little evidence available to assess the operation of sexual selection arising from mate searching.

Because females appear to influence pair formation, female preference may contribute to variance in male mating success and impose sexual selection on male traits. Female mate preferences may evolve when females gain direct fitness benefits as a result of mate choice. As in other amphipods, opportunities for direct benefits in *Hyalella* may occur as a consequence of precopulatory pairing, and effects arising from predation seem especially plausible because pairing influences predation risk (Cothran 2004). Given that individuals of both ecomorphs are subject to size-dependent mortality from predation (Fig. 7.3), a female's mortality risk may covary with the size of her mate because apparent size of the pair would depend in part on male size. Furthermore, because the direction of size-biased predation differs between ecomorphs, one could expect female preferences to be divergent between ecomorphs, with females of the large species preferring larger males, and females of the small species preferring smaller males. Although large-ecomorph females do prefer larger males, small-ecomorph females do not prefer smaller males, suggesting that this explanation may contribute to observed mating biases but cannot fully explain them.

Indirect selection, mediated through a correlation between preferred traits and offspring fitness (Kokko et al. 2003), may also contribute to evolution of mating biases in the ecomorphs. The qualitatively disparate mortality regimes experienced by large and small species suggests that mate size may correlate with offspring fitness. Because mortality declines with size in the large species, while competitive ability and foraging rate increase, as does mating success (Wellborn 1994, 1995, 2002), larger body size may indicate male genetic quality, provided size is heritable. In the small species, however, small or moderate size in males may indicate highest genetic quality due to the high mortality cost of large size. Alternatively, selection on preference for larger size may simply be relaxed in the small species because larger size is not associated with higher fitness. The disparity in mating biases observed between *Hyalella* ecomorphs is roughly consistent with

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evolution of mate preferences by indirect sexual selection acting across different regimes of natural selection, suggesting that further research is warranted.

Sexual Size Dimorphism

Ecomorph differences in the form of sexual selection acting on male body size can leave an evolutionary footprint in patterns of sexual size dimorphism (Andersson 1994). (p.161) Patterns of dimorphism in *Hyalella* are consistent with these disparate regimes of sexual selection. Large and small ecomorph species generally differ in the direction of sexual size dimorphism, with males being the larger sex in large ecomorph species, and the smaller sex in most small ecomorph species (Wellborn 1995, Wellborn and Cothran 2004, Wellborn et al. 2005). In Oklahoma, for example, large-species males are about 34% larger than females in mass, but males of the small species are 10% smaller than females (Wellborn and Bartholf 2005). Furthermore, as juveniles, males of the large ecomorph grow faster than females, contributing to species differences in adult sexual size dimorphism and suggesting strong selection for rapid growth in large-ecomorph males (Wellborn and Bartholf 2005). This interspecific variation in sexual size dimorphism can be driven by species disparity in female mate preference and thus may ultimately derive from the evolutionary effects of ecological constraints on sexual selection.
Future Directions
While it is evident that many characteristics of freshwater amphipods make them well suited as research models for understanding evolution of mating behavior, several challenges must be overcome before we can realize their full potential for informing current conceptual issues confronting the study of mating behavior and sexual selection. These hurdles largely parallel those facing the science as a whole (Kokko et al. 2003, Cordero and Eberhard 2003, Arnbjörnsson and Rowe 2005). First, it is essential that we better understand the roles of males and females in pair formation by disentangling female mate preference from male coercion, a difficult problem in many species (Bisazza et al. 2001, Cordero and Eberhard 2003, Kokko 2005). Such information is required because mechanisms of sexual selection, and selected traits will depend on the extent to which each sex exercises control over mating (Fincke 1997). Detailed behavioral studies and manipulative experiments can contribute substantially to our understanding of this issue (Jormalainen and Merilaita 1995, Sparkes et al. 2000), and studies empirically evaluating direct and indirect selection on female preference hold much potential for illuminating this difficult issue (Head et al. 2005). The related issue of confidently assessing paternity also deserves further attention, and molecular markers will be especially beneficial for evaluating this subject. Although takeovers are generally considered rare based on visual observations during the guarding phase, takeovers might be most likely during the narrow time widow of the female molt and copulation, especially if multiple copulation bouts are needed for successful fertilization (Hume et al. 2005).

Second, evaluating sexual selection on body size, and traits that covary with size, is hindered in amphipods by the natural correlation between size and age that is characteristic of indeterminate growth (Wellborn 1995). The correlation between size and age implies that observed mating biases do not directly estimate biases in lifetime mating success but does imply sexual selection is acting on body size to the extent that variation in male body size reflects variation in age-specific size. At minimum, we need to employ laboratory rearing studies to assess levels of variation in age-specific body size. A better method is to assess mating success across the lifetime of individuals, rather than during “snapshot” samples, but measurement of lifetime reproductive (p.162)
success is a seemingly impossible task in these small organisms living in complex environments. Molecular genetic methods, however, may hold much promise for allowing measurement of reproductive success and sexual selection under natural conditions (Ritland 1996, Gibbs and Weatherhead 2001). Such molecular methods will likely be essential for achieving substantial strides toward a broader understanding of sexual selection under natural conditions.

Finally, it is imperative that we examine the nature of direct and indirect benefits that can shape female mating preferences and male traits. Some progress will be relatively straightforward, such as assessing effects of male mate size on direct benefits accruing to females through changes in the female’s predation risk, feeding rate, or energetic costs, for example. Progress in understanding the nature and importance of indirect benefits of mate choice will be more challenging (Kokko et al. 2003), but laboratory studies assessing associations between female preference and offspring fitness are feasible (Hine et al. 2002, Head et al. 2005), and molecular genetic methods may allow such assessments under more natural conditions (Gibbs and Weatherhead 2001).
Summary and Conclusions
Although freshwater amphipods have been a research model for studies of male time-investment strategies during mating, much less is known about female mating preferences, the nature of benefits that drive female choice, and the influence of sexual conflict on female preference. Females may exercise mate choice by altering the form or intensity of resistance behavior. Direct selection on female mating preferences is likely to occur during the contact pairing phase because costs incurred or benefits gained by females during pairing are likely to depend on traits of guarding males. Indirect benefits from mate choice may also influence evolution of female preferences and male traits, but this has not been addressed empirically. Comparative studies of mating biases in interspecific ecomorphs in the genus *Hyalella* help to shed light on the evolution of female preference and preferred male traits. In species that occupy habitats where large body size is favored by ecological processes, females prefer larger males throughout the size range of males. For species subject to intense fish predation, however, mortality selection favors small body size, and female preference for larger males is weak, exhibiting a bias against the smallest males but indifference across intermediate to large male size. These divergent mating biases are consistent with direct and indirect selection on female preference acting under the disparate regimes of natural selection faced by the ecomorphs. Future advances in understanding the evolution of mating behavior in amphipods will require better integration of the joint roles of males and females in pairing, and mechanisms of sexual selection that drive mating biases.

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