

Size-assortative pairing across three developmental stages in the Zeus bug, *Phoreticovelia disparata*

Therésa M. Jones · Göran Arnqvist ·
Kathryn B. McNamara · Mark A. Elgar

Received: 18 November 2011 / Revised: 29 February 2012 / Accepted: 20 March 2012 / Published online: 4 April 2012
© Springer-Verlag 2012

Abstract The mechanisms underlying size-assortative pairing have received considerable attention. Typically, pairing is assumed to occur at, or just prior to, the adult phase of the life cycle. However, in many invertebrates, males commence associations with juvenile females who are more than a single moult away from sexual maturity. These species are ideal to explore the importance of reproductive and survival benefits as mechanisms driving size-assortative pairing. In the Zeus bug, *Phoreticovelia disparata*, adult males are found riding on juvenile (fourth and fifth instar) and adult females—a behaviour that is costly for females but has survival benefits for males. Using a combination of field collections and laboratory manipulations, we show that pairing is size-assortative both within and between female age classes and that riding males are smaller than non-riding males. In a series of mating trials, we revealed that males attempt to ride any female but that their riding success is dependent on female age. We also provide the first direct evidence of female resistance to male riding attempts in *P. disparata*. We propose that size-assortative pairing arises through adaptations that have evolved to minimise the

potential costs of sexual conflict. We suggest that the selective pressure on males to maximise survival benefits is sufficiently high that it outweighs the reproductive benefits of discriminating against fourth instar females. Finally, given that female resistance is under direct selection in juvenile females, it is likely to be the main form of selective pressure for adult females.

Keywords Size-assortative pairing · Developmental stages · Zeus bug · *Phoreticovelia disparata* · Juveniles

Introduction

Males and females of many species form associations that persist prior to and beyond the act of copulation (Andersson 1994; Simmons 2001; Arnqvist and Rowe 2005). These associations range from relatively brief periods of cohabitation to life-long pairings; they may be of mutual benefit or alternatively may benefit one sex to the detriment of the other, thus creating considerable conflict (Jormalainen 1998; Arnqvist and Rowe 2005; Parker 2006). In some species, there is a remarkable congruence of body size between pairs of males and females that result in size-assortative mating (Day and Butlin 1987; Otronen 1993; Miyashita 1994; Arnqvist et al. 1996; Rowe and Arnqvist 1996; Amano and Hayashi 1998; Johnson 1999; Masumoto 1999; Shine et al. 2001; Sutherland et al. 2007; Han et al. 2010). The underlying behavioural generators of size-assortative pairing have received considerable theoretical and empirical attention (Crespi 1989; Arnqvist et al. 1996; Rowe and Arnqvist 1996; Arnqvist 1997; Arnqvist et al. 1997; Harari et al. 1999; Fawcett and Johnstone 2003; Baldauf et al. 2009; Taborsky et al. 2009; Franceschi et al. 2010; Han et al. 2010). While size-assortative pairings may arise through

Communicated by D. Kemp

T. M. Jones (✉) · M. A. Elgar
Department of Zoology, The University of Melbourne,
Victoria 3010, Australia
e-mail: theresa@unimelb.edu.au

G. Arnqvist
Department of Ecology and Genetics,
Evolutionary Biology Centre, University of Uppsala,
Uppsala, Sweden

K. B. McNamara
School of Animal Biology, University of Western Australia,
Perth, WA 6009, Australia

mate choice by either or both sexes (sensu, Crespi 1989), they may also arise through intra-sexual competition if, for example, size determines when an individual has access to mates (Wong and Candolin 2005; Taborsky et al. 2009). Patterns of size-assortative mating may be further strengthened if mate choice targets condition-dependent traits, rendering only the highest quality (and potentially largest) individuals available for pairing (Harari et al. 1999; Fawcett and Johnstone 2003; Baldauf et al. 2009; Franceschi et al. 2010).

Size-assortative pairing may be an effective mechanism for minimising the costs imposed by sexual conflict (Fairbairn 1993; Harari et al. 1999; Arnqvist and Rowe 2005). This may be particularly pertinent in species where one sex, typically females, carries their mate for an extended period pre-, during or post-copulation. The costs of such associations can be high for both sexes. Guarding males may have a reduced feeding rate (Robinson and Doyle 1985; Sparkes et al. 1996), increased energy expenditure (Jormalainen and Merilaita 1993; Watson et al. 1998; Plaistow et al. 2003; Benesh et al. 2007), limited mating opportunities (Jormalainen et al. 1994a; Jormalainen 1998; Titelman et al. 2007; Cothran 2008) and an enhanced risk of predation (Ward 1986; Dick et al. 1995; Elgar and Fahey 1996; Cothran 2004) or injury (Benesh et al. 2007). For females, the presence of a guarding male may reduce development or survival (Jormalainen et al. 2001; Wedell et al. 2006; Jones et al. 2010), may impose increased energetic costs (Watson et al. 1998), decrease food intake rate (Arnqvist et al. 2006) and increase the risk of predation (Arnqvist 1989; Rowe 1994; Cothran 2004). At the extreme, males of some species may cannibalise their females (Ward 1986; Dick et al. 1993; Dick 1995). Females may tolerate male presence because guarding males shield them from costly harassment imposed by other males (Rowe 1994; Amano and Hayashi 1998; Watson et al. 1998). However, in those species where sexual conflict affects the mating system (and arguably, this may be the vast majority), conflict may promote the evolution of traits that enhance pairing success (typically in males) and of resistance traits (typically in females) that increase the ability to resist pairing attempts (for a comprehensive review, see Arnqvist and Rowe 2005). Such a scenario can lead to size-assortative pairing.

Previous studies have sought the causes of size-assortative mating exclusively in the adult phase of the life cycle, yet pairing commences during the juvenile phase of the female life cycle in many invertebrates (Slooten and Lambert 1983; Burton 1985; Boxshall 1990; Evstigneeva 1993; Durbaum 1995; Ritchie et al. 1996; Fiers 1998; Jormalainen 1998; Thiel 2002; Zhu and Tanaka 2002; Arakaki et al. 2004; Bel-Venner and Venner 2006; Oku 2009). Associations between adult males and juvenile females have substantial consequences for both sexes. Moreover, the selective pressures promoting size-assortative mating are likely to vary with female

developmental stage. From a female perspective, juvenile females are relatively smaller than adult females. Thus, the net costs outlined previously are likely to be much greater, particularly in species where females physically carry a male. For males, pairing with a juvenile female may reduce energetic costs, but it yields no immediate reproductive benefits unless she reaches sexual maturity during this period (Parker 1974; Grafen and Ridley 1983; Jormalainen 1998). Such species provide useful model systems as they can provide insights into whether patterns of size-assortative pairing are driven by reproductive and/or survival costs across or within the sexes.

The semi-aquatic Zeus bug, *Phoreticovelia disparata*, is extremely sexually size-dimorphic (Andersen and Weir 2001). The smaller adult males ride on the back of females (using their legs to secure and maintain a position), and this pre- and post-mating association may last several days (Arnqvist et al. 2007). The operational sex ratio is typically male-biased (Arnqvist et al. 2007). Adult males commence riding on the backs of juvenile fourth (penultimate) instar females, and some males may remain with their female through the fifth instar to the adult stage, although it is likely that many males switch partners during this period (Arnqvist et al. 2007). Males never attempt to ride females younger than the fourth instar (TMJ personal observations). Riding males derive direct benefits from their associations with females. In addition to minimising the energetic costs of locomotion, males kleptoparasitise the prey captured by their female partners (Arnqvist et al. 2006). From the fourth instar, females are equipped with a pair of dorsal glands that produce a wax-like secretion (Andersen and Weir 2001; Arnqvist et al. 2003) which males consume when riding (Arnqvist et al. 2003) and which potentially benefits a female as its presence reduces male kleptoparasitic behaviour (Arnqvist et al. 2006). Despite the survival benefits, a male may accrue while riding a fourth instar female; he yields no reproductive benefits unless he remains with her until she is sexually mature. This suggests that there should be strong selection for males to preferentially ride fifth instar females, particularly those females that are closest to sexual maturity (sensu, Parker 1974; Grafen and Ridley 1983), or virgin adult females (because females are polyandrous). Whether Zeus bug males have evolved traits that increase the likelihood of pairing with a particular female is unknown.

For juvenile females, the presence of a riding male is costly: Fourth instar females with a riding male are less likely to survive through to the adult phase of the life cycle, and those that do survive have reduced adult longevity (Jones et al. 2010). The benefits, if any, of having a male present during juvenile development are unclear. Because females can store sperm for up to 3 weeks (Arnqvist et al. 2003), natural populations are dense and show a male-biased operational sex ratio (Arnqvist et al. 2007), it is

unlikely that riding males represent a valuable insurance against a shortage of viable sperm. Combined, these data suggest that selection should favour young juvenile females to either avoid having riding males or at least minimise the costs associated with bearing a male, perhaps by ensuring that the male is smaller than the population average, or by more vigorous resistance in attempts by larger males to pair. Moreover, given that females spend the majority of their adult lives paired with a male, they may also benefit from being paired with a smaller individual. Accordingly, we predict size-assortative pairing both within and across the three female developmental stages.

The aim of this study was threefold. First, we explored the degree of size-assortative mating under natural conditions by collecting paired and unpaired adult females directly from the field. Second, we assessed the degree of size-assortative pairings of males with fourth instar, fifth instar and adult females. Finally, we determined experimentally whether the female instars varied in their ability to resist the riding attempts of adult males by conducting mating trials in which a single male was placed with three females (one fourth instar juvenile, one fifth instar juvenile and one adult female) and observing the frequency of successful and unsuccessful riding attempts.

Materials and methods

Field samples

Adult morphology and size-assortative mating under natural conditions

Zeus bugs *P. disparata* (Heteroptera; Gerridae) were collected from three sites (approximately 200 m apart) along the Little Mulgrave River, Queensland, Australia (immediately upstream from the Mulgrave River (17° 7' 60 s; 145° 43' 60 e)) in September 2008. Pairs of adult females with riding males and solitary males were collected using a sweeping technique with a hand net (Arnqvist et al. 2007). We never observed females without a riding male and thus could not collect solitary females (sensu, Arnqvist et al. 1996). Each riding pair ($N=26$ pairs; Table 1) was transferred immediately to a single 1.7-ml Eppendorf tube containing 70 % alcohol; single males were placed collectively into a single tube ($N=36$; Table 1). We measured several aspects of size. First, we removed the left second leg from each individual, mounting it on a slide in a small drop of glycerol and then flattening it using a glass coverslip. Leg length was measured from the left second leg, unless this was damaged, in which case, the right leg was used ($N=2$ females; 2 males). We used the second leg as there was a strong correlation between the length of a male's first and second legs ($r=0.89$, $N=61$ males, $P<0.0001$). Second, we

measured the body of each specimen (from the tip of the first to the final abdominal tergite). We captured digital images of the leg (Olympus BX51; magnification $\times 100$) and the body (Olympus SZX7; magnification $\times 40$) and then measured the digitised images using ImageJ 1.41 (NIH, USA). To confirm their adult status, we subsequently dissected and then examined all females for the presence of stored eggs.

Laboratory trials

Individuals contributing to our laboratory trials were collected from the Little Mulgrave River (site 3) and were brought into the laboratory to form a stock population (approximately 1,500 individuals). Bugs were maintained in aerated 30 \times 40 cm tanks (water depth 10 cm), provided with polystyrene blocks and strips of balsa wood (as resting and oviposition sites) and fed *ad libitum* food (frozen cricket nymphs, *Acheta domesticus* and adult *Drosophila melanogaster*). All individuals used in the experiment were of the apterous morph (Andersen and Weir 2001).

Adult morphology and size-assortative pairing across female instars

The relative size of males riding on fourth, fifth and adult females was assessed by collecting females from the field and maintaining them in standard culture conditions for 24 h. This method was used in preference to collecting pairs straight from the field because most fourth and some fifth instar females lost their male during the capture process (TMJ personal observations), and thus our field samples may be biased. We maintained individuals in one large culture (approximately 1,500 individuals) for 24 h and then selected haphazardly 15 fourth instar females, 15 fifth instar females and 15 adult females with their associated riding male. We killed the pair immediately by placing them in 70 % alcohol. For laboratory specimens, we took leg and body measurements (as above) to assess whether male body size also varied with female age class. To confirm their adult status, we subsequently dissected and then examined all females for the presence of stored eggs. Juvenile females are also distinguished by a reduced number of tarsomeres on their tarsi.

Female developmental stage and male pairing behaviour

To explore whether female instar had any impact on the outcome of male riding behaviour, we separated males and females (fourth and fifth instar and adults) by sex for a 24-h period. Individuals were maintained in single sex holding containers that provided the same conditions as the stock population. After 24 h, 35 groups each consisting of three

Table 1 Mean (\pm standard error) leg and body lengths for single males and adult riding pairs of the Zeus bug, *P. disparata*

	Female	Riding males	Single males	Comparison between riding and single males
Body length (mm)				
Site 1	1.505 \pm 0.018	0.753 \pm 0.014	–	Effect of riding, $F_{1,45}=338.5$, $P<0.0001$
Site 2	1.591 \pm 0.018	0.707 \pm 0.027	0.962 \pm 0.014	Effect of site, $F_{1,45}=5.99$, $P=0.02$
Site 3	1.522 \pm 0.016	0.746 \pm 0.011	0.990 \pm 0.007	Riding \times site, $F_{1,45}=0.20$, $P=0.66$
Leg length (mm)				
Site 1	0.550 \pm 0.005	0.394 \pm 0.002	–	Effect of riding, $F_{1,45}=3.09$, $P=0.08$
Site 2	0.554 \pm 0.010	0.390 \pm 0.005	0.362 \pm 0.007	Effect of site, $F_{1,45}=2.12$, $P=0.15$
Site 3	0.547 \pm 0.006	0.382 \pm 0.006	0.388 \pm 0.003	Riding \times site, $F_{1,45}=7.74$, $P=0.01$

Samples were collected from three sites (site 1, $n=0$ single males and ten riding pairs; site 2, $n=16$ single males and six riding pairs; site 3, $n=20$ single males and ten riding pairs) along the Little Mulgrave River in Queensland, Australia

females (one fourth instar, one fifth instar and one adult instar) and a single adult male were selected at random from the holding containers. Each group of three females was placed immediately into a small Petri dish (depth=12 mm; width=38 mm) containing 30 ml water (approximately 3 mm depth). Females were left for 3 min to acclimate, which was sufficient time for them to either rest on the side of the dish or remain relatively still on the water surface. After this period, a single adult male was introduced to the centre of the dish and left for 10 min or until he successfully established a secure riding position on one of the three females' backs. We denoted a successful riding event as one where the female ceased to display resistance behaviour. As in most semi-aquatic Heteroptera (see Arnqvist 1997), female Zeus bugs resist male pairing attempts. Females attempt to dislodge the male with her mid- and hind legs immediately following a riding attempt and prior to him obtaining a secure grip on her back. In many cases, this was combined with females turning over onto their backs in the water and using their legs to dislodge the male. For each trial, we also recorded the presence and duration of all male approach and female resistance behaviours to male riding attempts.

Statistics

Data from the field samples and the choice assays were analysed using JMP version 8.0 (2009 SAS Institute Inc., Cary, NC, USA). Morphological comparisons across the female stages were made using ANOVAs in SYSTAT (version 13). In cases where the overall ANOVA was significant (P_{ANOVA}), the shape of the response across female stages was characterised with polynomial post hoc contrasts. First-order polynomial contrasts (P_{1st}) tested whether the response was linear over female stages and the second-order polynomial contrasts (P_{2nd}) tested whether the response was significantly non-linear over female stages. In no case were

variances significantly heterogenous (Levene's test) or residuals significantly non-normal (Shapiro–Wilk's test). Five pairs were removed from the analyses of laboratory body size correlations. In three cases, one of the two specimens within a pair was damaged (one fourth instar and two fifth instar pairs). In two further cases, the female from the fifth instar pair was subsequently discovered to be an adult female.

Results

Field-caught adults

Adult morphology and size-assortative mating under natural conditions

After controlling for site, we found evidence for strong size-assortative pairing between adult males and females with respect to body length ($F_{1,20}=12.11$, $P=0.002$; Fig. 1a) but limited evidence for a correlation between male and female leg length ($F_{1,20}=2.73$, $P=0.11$, Fig. 1b).

We obtained no single males from Site 1 and thus first compared morphological characteristics of riding males only (from all sites). There was no variation in male body size ($F_{2,23}=1.99$, $P=0.16$; Table 1) male leg length ($F_{2,23}=2.09$, $P=0.15$; Table 1) or the ratio between leg and body length ($F_{2,23}=3.04$, $P=0.07$; Table 1) of riding males across the three sites. A comparison of riding and single males (from sites 2 and 3) revealed that riding males had significantly smaller bodies than single males but equally long legs (Table 1). However, there was also considerable inter-site variation (Table 1).

All adult females had eggs present in their abdomens. Female body length varied ($F_{2,23}=5.57$, $P=0.01$; Table 1), but female leg length was comparable ($F_{2,23}=0.17$, $P=0.85$; Table 1) across the three sites.

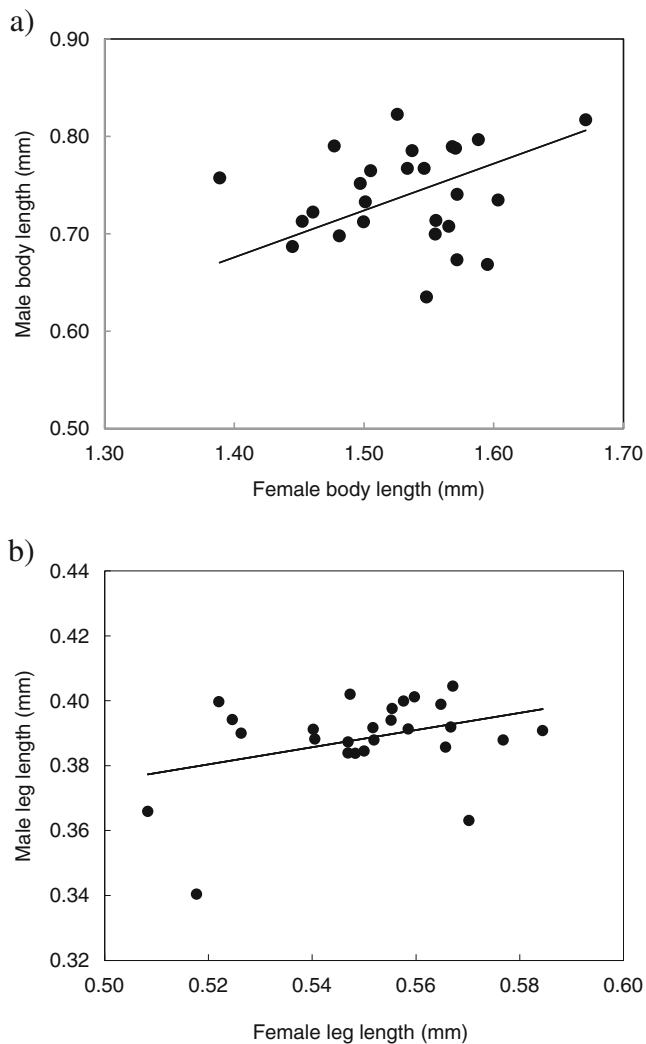


Fig. 1 Relationship between **a** male and female body length (millimetres) ($y=+0.002+0.48x$, $r^2=0.98$) and **b** male and female leg length (millimetres) ($y=0.24+0.26x$, $r^2=0.13$) for field-caught males riding adult females from the Little Mulgrave river (Queensland)

Laboratory trials

Adult morphology and size-assortative pairing across female instars

Our laboratory trials provided evidence for size-assortative pairing across the three female instars with respect to leg length ($F_{1,36}=5.74$, β [SE]=0.26 [0.11], $P=0.02$; Fig. 2) but not body length ($F_{1,36}=0.24$, β [SE]=−0.06 [0.12], $P=0.62$). These patterns were consistent across instars (effect of instar \times size in both models, $P>0.23$).

Male leg length increased linearly across the three female instars (Table 2). In contrast, male body length was comparable across the three female instars (Table 2).

All sampled adult (but no fourth or fifth instar) females had eggs retained in their abdomen (mean \pm SE number of

eggs= 4.13 ± 0.37). There was no relationship between female body size and the number of eggs retained (Spearman rank correlation, $r_s=0.02$, $P=0.95$). Female size (leg and body length) increased linearly between the fourth instar to the adult stage of the life cycle (Table 2).

Adult males were smaller (both body and leg length) than their female mate, regardless of her instar (paired t tests within each instar, all $P<0.001$; Table 2). However, a comparison between the leg to body ratios of males and females revealed that males had relatively longer legs compared with females (paired t test, $t=6.62$, $df=39$, $P<0.0001$; Table 2).

Female developmental stage and male pairing behaviour

Males attempted to ride at least one of the three females in 34 of the 35 trials (Fig. 3). Five trials that did not result in a male successfully riding a female were discarded from statistical analyses. In one trial, the male sat on the side of the dish and did not approach any female; in three trials, the male repeatedly attempted, but failed, to secure a riding position on any of the three females and in the fifth trial, the male attempted to ride the fourth instar female but she struggled violently, repeatedly turning over in the water in an attempt to dislodge him with her legs. The male persisted in his attempts, and she eventually drowned after 173 s, at which time the male dismounted.

Females usually struggled violently following a male's initial attempt to ride. We observed only five (of 58) instances where the male approached and commenced riding a female without her exhibiting any overt resistance behaviour (one fourth instar; three fifth instar and one adult female). All failed riding attempts ($N=28$) were apparently due to the

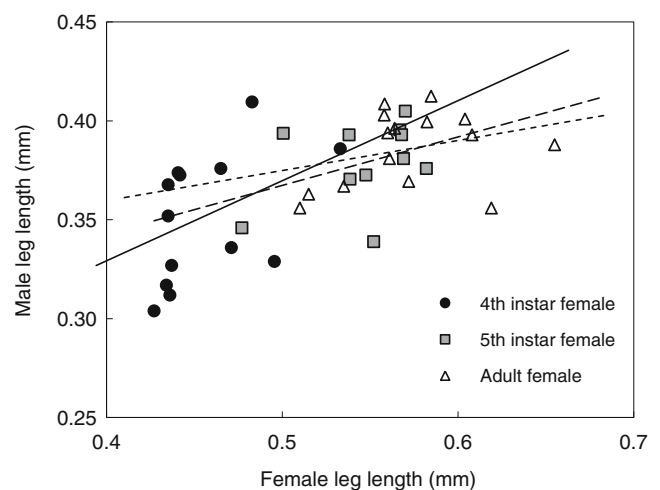


Fig. 2 Relationship between male and female leg length (millimetres) for males paired to fourth instar (solid line, $y=0.17+0.40x$, $r^2=0.20$), fifth instar (large dash line, $y=0.25+0.24x$, $r^2=0.14$) and adult (small dash line, $y=0.15x+0.30$, $r^2=0.09$) females

Table 2 Morphological measurements for fourth ($N=14$), fifth ($N=11$) and adult ($N=15$) females and their associated males

Lengths (mm)	Fourth instar	Fifth instar	Adult	$F_{2,37}$	P_{ANOVA}	P_{1st}	P_{2nd}
Female body length	1.65±0.02	2.038±0.02	2.11±0.02	128.00	<0.001	<0.001	<0.001
Female leg length	0.45±0.01	0.55±0.01	0.57±0.01	44.85	<0.001	<0.001	0.010
Male body length	1.22±0.02	1.28±0.02	1.26±0.02	3.19	0.053	–	–
Male leg length	0.35±0.01	0.38±0.01	0.39±0.01	8.27	0.001	<0.001	0.230

Individuals were selected at random from a large laboratory population (N =approximately 1,500) that had been maintained under standard conditions for 24 h. Comparisons between the female stages were made using ANOVAs. In cases where the overall ANOVA was significant (P_{ANOVA}), the shape of the response across female stages was characterised with polynomial post hoc contrasts. Here, first-order polynomial contrasts (P_{1st}) test whether the response was linear over female stages and the second-order polynomial contrasts (P_{2nd}) test whether the response was significantly non-linear over female stages. In no case were variances significantly heterogenous (Levene's test) or residuals significantly non-normal (Shapiro–Wilk's test)

female struggling violently and dislodging the male. While violent, struggles were typically short. Struggle duration varied across the three female instars ($F_{2,54}=4.48$, $P=0.017$; Fig. 4) but was not related to the success of the riding attempt ($F_{1,54}=2.36$, $P=0.13$; interaction between female and the success of the riding attempt, $F_{2,54}=0.83$, $P=0.44$). Following the initial struggling period, females with riding males ceased struggling and appeared relatively acquiescent.

In the majority of the 30 trials where a male successfully remained on the female, the male approached and attempted to ride only one of the three females ($N=19$ trials). Males were less likely to secure a successful riding position on fourth instar females ($N=4$ trials) than on either fifth instar females ($N=14$ trials; fourth vs fifth instar females, $\chi^2=5.2$, $P=0.02$) or adult females ($N=12$ trials; fourth vs adult females, $\chi^2=4.0$, $P=0.04$). This did not arise because of variation in male approach behaviour: The first female a male attempted to ride was equally likely to be a fourth instar ($N=9$ trials), fifth instar (ten trials) or adult female (11 trials).

Moreover, males were equally likely to attempt to commence riding on fourth ($N=18$), fifth ($N=18$) and adult instars ($N=24$; $F_{2,27}=1.39$, $P=0.27$). However, the probability of a riding event being successful varied significantly with female instar (nominal logistic model, $\chi^2=15.94$, $P=0.0003$; Fig. 3); pairing occurred more often with fifth instar females and least often with fourth instar females.

Discussion

We provide several lines of evidence for size-assortative pairing in the Zeus bug, *P. disparata*, both within and between juvenile female instars. Significantly, these data suggest that the selective pressures maintaining pairs are similar across juvenile and adult female instars. There were, however, also differences across stages and environments. Under natural conditions, adult males and females paired assortatively with respect to body but not leg length.

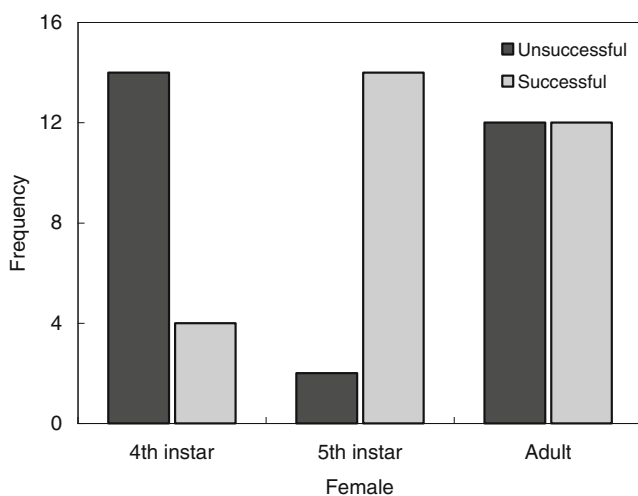


Fig. 3 The frequency of unsuccessful and successful male riding attempts for males attempting to ride fourth instar, fifth instar and adult females

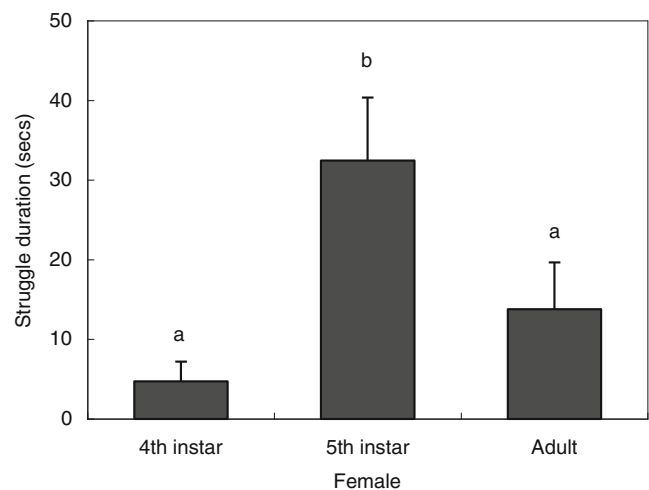


Fig. 4 The duration of successful struggles when males attempt to ride fourth, fifth and adult females. Error bars represent standard errors about the mean. Different letters denote significant differences between the three categories

Moreover, riding males had significantly smaller bodies than single (non-riding) males, although their legs were comparable in length. Investigation of initial pairing patterns under laboratory conditions revealed a positive linear relationship between male leg (but not body) length and female developmental stage. Finally, exploration of the pairing success of a single male with fourth and fifth juvenile and adult females provides the first direct evidence of female resistance behaviour in this system. Males were equally likely to approach any of the three instars and were more likely to be successful with fifth instar females and least successful with fourth instar females. Our choice assay further highlighted a substantial cost of resistance behaviour for fourth instar females which may explain the high incidence of mortality documented previously for this age class (Jones et al. 2010). We discuss our findings in relation to the evolution and maintenance of male riding behaviour in Zeus bugs.

Mechanism of size-assortative pairing across female instars

For males, the factors limiting size-assortative pairing are likely to vary across the three female instars. Our study indicates that the observed patterns are not driven by a male's preference for a particular-sized female (c.f. Beeching et al. 2004), and, given that they attempted to pair with all females, it is unlikely they are able to discern female age or sexual receptivity. It is also unlikely that intra-sexual competition prevented male access to particular females (sensu, Fawcett and Johnstone 2003; Hardling and Kokko 2005). Instead, we suggest that size-assortative pairing across the female age classes may arise because a male's leg length limits the size of a female he can successfully establish a riding position upon during the initial phase of female resistance. While males are always smaller than their mate, their relatively longer legs enable them to maintain a stable position on a female's back. However, the reduced body size (and presumably circumference) of fourth instar females means that, from a purely mechanical perspective, a male with a smaller leg length will be better able to obtain an initial leg-hold. The ability of a male to rapidly secure a riding position may also be of significance during kleptoparasitism—a key behaviour adopted by riding males (Arnqvist et al. 2006). To kleptoparasitise a prey item, a male leaves the females' dorsum and leans over her head. After feeding, he returns to his position on her dorsum during which time females can (and do) struggle (TMJ personal observations). Males that have legs most mismatched to their given females' body size, particularly when she is very small, may be less able to endure these initial resistance periods (Jormalainen et al. 1994b). Such a mismatch would explain why males attempting to ride fourth instar females were least successful at securing a riding position.

Although leg-length is likely to be important for a male to secure an initial position on a female Zeus bug, female

selective pressures may limit the duration of a male riding event. Ultimately, females should prefer a smaller (and presumably lighter) male as a riding partner as these should be energetically least costly to bear. Our field data confirm this prediction—riding males were smaller than single non-riding males. Combined, our data are consistent with a scenario in which there are opposing selective pressures on male leg and body length. Males have evolved significantly smaller bodies to ensure that they increase the likelihood of maintaining a position on a female but have maintained relatively longer legs to ensure they can secure a position during the initial pairing phase when females resist their presence most strongly.

Why is riding maintained?

The riding behaviour observed in *P. disparata* is unlikely to be mutually beneficial for both sexes (sensu, Crespi 1989). Indeed, there is clear evidence of sexual conflict in this system. For males, the benefits of riding any female from the fourth instar up are clear: they derive direct benefits either through consuming the waxy dorsal secretion produced by females (Arnqvist et al. 2003) or by kleptoparasitising the female's prey items (Arnqvist et al. 2006)—both increasing their survival prospects (Arnqvist et al. 2003). However, riding can potentially impose costs on males. Most obvious is the loss of fertilisation opportunities by riding fourth instar females, especially because he is unlikely to remain with her until she reaches sexual maturity (Jones et al. 2010). Despite this, males do not discriminate against fourth instar females nor, as our study shows, do they prefer late fifth instar females, theoretically the most valuable instar (Parker 1974; Grafen and Ridley 1983). Thus, while the reproductive benefits are likely to be limited, we suggest that the selective pressure on males to gain direct benefits and reduce the energetic costs of locomotion are sufficiently high that securing a riding position on any defendable female (sensu Venner et al. 2010), even one of lower quality, may be better than securing no female at all. The fact that operational sex ratios are generally strongly male-biased (Arnqvist et al. 2007) makes this scenario more likely. That a male sometimes continues to remain with a female until her death also lends additional support to this idea.

In contrast to males, the evidence collected thus far suggests that female Zeus bugs derive few, if any, benefits from the presence of a riding male, but, rather, they (particularly fourth instar females) incur considerable feeding and longevity costs (Arnqvist et al. 2003, 2006, 2007; Jones et al. 2010). Moreover, females are not sperm limited (Arnqvist et al. 2003), so even if there was a correlation between male size and fertilisation capability, it is unlikely to provide a sufficiently strong selective force to promote female preference for a riding male. Instead, we suggest that size-assortative pairing, preference for smaller riding males and the production of a waxy dorsal secretion (Arnqvist et al. 2006) have evolved in

females to mitigate the potential costs of a riding male, rather than provide some mutual benefit.

Why do females resist?

The resistance behaviour displayed by female Zeus bugs is potentially costly but is commonly observed in many taxa (Arnqvist and Rowe 2005). For example, adult female water striders somersault and roll in the water to prevent males from securing a riding position. Such struggles may last a few seconds (comparable to the durations observed in Zeus bugs) but may extend for several minutes in some species (Arnqvist 1997). There is considerable controversy over whether such female resistance is primarily under direct selection to reduce mating/pairing rate or under indirect selection to assess the genetic quality of males (i.e., “mate screening”) (Eberhard 2002; Arnqvist and Rowe 2005; Maklakov and Arnqvist 2009; Blyth and Gilburn 2011). Our study provides an entirely novel and very important clue to this debate. While “mate screening” may help explain why adult (and potentially also fifth instar) Zeus bug females struggle, it cannot add to the maintenance of resistance behaviour in fourth instar females. This is simply because the current riding male is very unlikely indeed to be one of her future mates. Instead, we suggest that juvenile females struggle simply to (1) avoid as many male pairing attempts as possible and (2) possibly to ensure that only the smallest and lightest males remain, thus minimising the potential costs of bearing males for extended periods. The fact that female resistance must be under direct selection in juvenile females suggest that this is also the main form of selection upon the very same female resistance behaviours in adult females.

Our study adds to a number of studies of insects in which overt male–female struggles result in size-assortative mating (Day and Butlin 1987; Arnqvist et al. 1996; Rowe and Arnqvist 1996; Blyth and Gilburn 2011). In addition, the fact that female resistance and size-assortment also occurred in juvenile females strongly implies that female resistance is primarily under direct selection to reduce the costs of interacting with males and that, consequently, size-assortative mating in these systems is best seen as a side-effect of overt sexual conflict over pairing.

Acknowledgements TMJ was funded by an Australian Research Council grant (DP0558265) and GA by the Swedish Research Council and the European Research Council.

References

- Amano H, Hayashi K (1998) Costs and benefits for water strider (*Aquarius paludum*) females of carrying guarding, reproductive males. *Ecol Res* 13(3):263–272
- Andersen NM, Weir TA (2001) New genera of Veliidae (Hemiptera: Heteroptera) from Australia, with notes on the generic classification of the subfamily Microveliinae. *Invert Taxon* 15(2):217–258
- Andersson M (1994) Sexual selection. Princeton, Princeton
- Arakaki N, Kishita M, Nagayama A, Fukaya M, Yasui H, Akino T, Hirai Y, Wakamura S (2004) Pre-copulatory mate guarding by the male green chafer, *Anomala albopilosa sakishimana* Nomura (Coleoptera: Searabaeidae). *Appl Entomol Zool* 39(3):455–462
- Arnqvist G (1989) Multiple mating in a water strider—mutual benefits or intersexual conflict. *Anim Behav* 38:749–756
- Arnqvist G (1997) The evolution of water strider mating systems: causes and consequences of sexual conflicts. In: Choe JC, Crespi BJ (eds) The evolution of mating systems in insects and arachnids. Cambridge University Press, Cambridge, pp 146–163
- Arnqvist G, Rowe L (2005) Sexual conflict. Monographs in behavior and ecology. Princeton University Press, New Jersey
- Arnqvist G, Rowe L, Krupa JJ, Sih A (1996) Assortative mating by size: a meta-analysis of mating patterns in water striders. *Evol Ecol* 10(3):265–284
- Arnqvist G, Thornhill R, Rowe L (1997) Evolution of animal genitalia: morphological correlates of fitness components in a water strider. *J Evol Biol* 10(4):613–640
- Arnqvist G, Jones TM, Elgar MA (2003) Insect behaviour: reversal of sex roles in nuptial feeding. *Nature* 424(6947):387–387
- Arnqvist G, Jones TM, Elgar MA (2006) Sex-role reversed nuptial feeding reduces male kleptoparasitism of females in Zeus bugs (Heteroptera: Veliidae). *Biol Lett* 2(4):491–493
- Arnqvist G, Jones TM, Elgar MA (2007) The extraordinary mating system of Zeus bugs (Heteroptera: Veliidae: *Phoreticovelina* sp.). *Aus J Zool* 55(2):131–137
- Baldauf SA, Kullmann H, Schroth SH, Thunken T, Bakker TCM (2009) You can't always get what you want: size assortative mating by mutual mate choice as a resolution of sexual conflict. *Bmc Evol Biol* 9:article 129
- Beeching SC, Wack CL, Ruffner GL (2004) Female convict cichlids (*Archocentrus nigrofasciatus*) prefer to consort with same-sized males. *Ethol Ecol Evol* 16:209–216
- Bel-Venner MC, Venner S (2006) Mate-guarding strategies and male competitive ability in an orb-weaving spider: results from a field study. *Anim Behav* 71:1315–1322
- Benesh DP, Valtonen ET, Jormalainen V (2007) Reduced survival associated with precopulatory mate guarding in male *Asellus aquaticus* (Isopoda). *Ann Zool Fenn* 44(6):425–434
- Blyth J, Gilburn A (2011) The function of female behaviours adopted during premating struggles in the seaweed fly, *Coelopa frigida*. *Anim Behav* 81:77–82
- Boxshall GA (1990) Precopulatory mate guarding in copepods. *Bijdr Tot Dierkunde* 60(3–4):209–213
- Burton RS (1985) Mating system of the intertidal copepod *Tigriopus californicus*. *Mar Biol* 86(3):247–252
- Cothran RD (2004) Precopulatory mate guarding affects predation risk in two freshwater amphipod species. *Anim Behav* 68:1133–1138
- Cothran RD (2008) Phenotypic manipulation reveals sexual conflict over precopula duration. *Behav Ecol Sociobiol* 62(9):1409–1416
- Crespi BJ (1989) Causes of assortative mating in arthropods. *Anim Behav* 38:980–1000
- Day TH, Butlin RK (1987) Non-random mating in natural populations of the seaweed fly, *Coelopa frigida*. *Heredity* 58:213–220
- Dick JTA (1995) The cannibalistic behavior of 2 *Gammarus* species (Crustacea, Amphipoda). *J Zool* 236:697–706
- Dick JTA, Montgomery I, Elwood RW (1993) Replacement of the indigenous amphipod *Gammarus duebeni celticus* by the introduced *Gammarus pulex*—differential cannibalism and mutual predation. *J Anim Ecol* 62(1):79–88
- Dick JTA, Elwood RW, Montgomery WI (1995) The behavioral basis of a species replacement—differential aggression and predation

- between the introduced *Gammarus pulex* and the Native *G duebeni celticus* (Amphipoda). Behav Ecol Sociobiol 37(6):393–398
- Durbaum J (1995) Discovery of postcopulatory mate guarding in *Copepoda harpacticoida* (Crustacea). Mar Biol 123(1):81–88
- Eberhard W (2002) The function of female resistance behavior: Intromission by male coercion vs. female cooperation in sepsid flies (Diptera: Sepsidae). Rev Biol Trop 50(2):485–505
- Elgar MA, Fahey BF (1996) Sexual cannibalism, competition, and size dimorphism in the orb-weaving spider *Nephila plumipes* Latreille (Araneae: Araneoidea). Behav Ecol 7(2):195–198
- Evstigneeva TD (1993) Precopulatory mate guarding in *Harpacticella inopinata* Sars (Copepoda, Harpacticoida) from Lake Baikal. Hydrobiologia 254(2):107–110
- Fairbairn DJ (1993) Costs of loading associated with mate carrying in the waterstrider, *Aquarius remigis*. Behav Ecol 4(3):224–231
- Fawcett TW, Johnstone RA (2003) Mate choice in the face of costly competition. Behav Ecol 14(6):771–779
- Fiers F (1998) Female leg 4 development in Laophontidae (Harpacticoida): a juvenile adaptation to precopulatory behaviour. J Mar Sys 15(1–4):41–51
- Franceschi N, Lemaitre JF, Cezilly F, Bollache L (2010) Size-assortative pairing in *Gammarus pulex* (Crustacea: Amphipoda): a test of the prudent choice hypothesis. Anim Behav 79(4):911–916
- Grafen A, Ridley M (1983) A model of mate guarding. J Theor Biol 102:549–567
- Han CS, Jablonski PG, Kim B, Park FC (2010) Size-assortative mating and sexual size dimorphism are predictable from simple mechanics of mate-grasping behavior. BMC Evol Biol 10:359
- Harari AR, Handler AM, Landolt PJ (1999) Size-assortative mating, male choice and female choice in the curculionid beetle *Diaeprepes abbreviatus*. Anim Behav 58:1191–1200
- Hardling R, Kokko H (2005) The evolution of prudent choice. Evol Ecol Res 7(5):697–715
- Johnson LJ (1999) Size assortative mating in the marine snail *Littorina neglecta*. J Mar Biol Assoc UK 79(6):1131–1132
- Jones TM, Elgar MA, Arnqvist G (2010) Extreme cost of male riding behaviour for juvenile females of the Zeus bug. Anim Behav 79(1):11–16. doi:10.1016/j.anbehav.2009.10.016
- Jormalainen V (1998) Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. Quart Rev Biol 73:275–304
- Jormalainen V, Merilaita S (1993) Female resistance and precopulatory guarding in the isopod *Idotea baltica* (Pallas). Behaviour 125:219–231
- Jormalainen V, Merilaita S, Tuomi J (1994a) Male choice and male-male competition in *Idotea baltica* (Crustacea, Isopoda). Ethology 96(1):46–57
- Jormalainen V, Tuomi J, Merilaita S (1994b) Effect of female resistance on size-dependent precopula duration in mate-guarding crustacea. Anim Behav 47(6):1471–1474
- Jormalainen V, Merilaita S, Riihimäki J (2001) Costs of intersexual conflict in the isopod *Idotea baltica*. J Evol Biol 14(5):763–772
- Maklakov A, Arnqvist G (2009) Testing for direct and indirect effects of mate choice by manipulating female choosiness. Curr Biol 19:1903–1906
- Masumoto T (1999) Size assortative mating and reproductive success of the funnel-web spider, *Agelena limbata* (Araneae: Agelenidae). J Insect Behav 12(3):353–361
- Miyashita T (1994) Size-related mating and mate guarding in the orb-web spider *Nephila clavata* (Araneae, Araneidae). J Insect Behav 7(3):289–296
- Oku K (2009) Female mating strategy during precopulatory mate guarding in spider mites. Anim Behav 77(1):207–211
- Otronen M (1993) Size assortative mating in the yellow dung fly *Scatophaga stercoraria*. Behaviour 126:63–76
- Parker GA (1974) Courtship persistence and female guarding as male time investment strategies. Behaviour 48:157–184
- Parker GA (2006) Sexual conflict over mating and fertilization: an overview. Phil Trans R Soc B 361:235–259
- Plaistow SJ, Bollache L, Cezilly F (2003) Energetically costly precopulatory mate guarding in the amphipod *Gammarus pulex*: causes and consequences. Anim Behav 65:683–691
- Ritchie G, Mordue AJ, Pike AW, Rae GH (1996) Observations on mating and reproductive behaviour of *Lepeophtheirus salmonis*, Kroyer (Copepoda: Caligidae). J Exp Mar Biol Ecol 201(1–2):285–298
- Robinson B, Doyle R (1985) Trade-off between male reproduction (amplexus) and growth in the amphipod *Gammarus lawrencianus*. Biol Bull 168:482–488
- Rowe L (1994) The costs of mating and mate choice in water striders. Anim Behav 48(5):1049–1056
- Rowe L, Arnqvist G (1996) Analysis of the causal components of assortative mating in water striders. Behav Ecol Sociobiol 38(4):279–286
- Shine R, O'Connor D, Lemaster MP, Mason RT (2001) Pick on someone your own size: ontogenetic shifts in mate choice by male garter snakes result in size-assortative mating. Anim Behav 61:1133–1141
- Simmons LW (2001) Sperm competition and its evolutionary consequences in the insects. Princeton University Press, New Jersey
- Slouten E, Lambert DM (1983) Evolutionary studies of the New Zealand coastal mosquito *Opifex fuscus* (Hutton). I. Mating behavior. Behaviour 84:157–172
- Sparkes TC, Keogh DP, Pary RA (1996) Energetic costs of mate guarding behavior in male stream-dwelling isopods. Oecologia 106(2):166–171
- Sutherland DL, Hogg ID, Waas JR (2007) Is size assortative mating in *Paracalliope fluviatilis* (Crustacea: Amphipoda) explained by male-male competition or female choice? Biol J Linn Soc 92(1):173–181
- Taborsky B, Guyer L, Taborsky M (2009) Size-assortative mating in the absence of mate choice. Anim Behav 77(2):439–448
- Thiel M (2002) Reproductive biology of a small isopod symbiont living on a large isopod host: from the maternal marsupium to the protective grip of guarding males. Mar Biol 141(1):175–183
- Titelman J, Varpe O, Eliassen S, Fiksen O (2007) Copepod mating: chance or choice? J Plankton Res 29(12):1023–1030
- Venner S, Bernstein C, Dray S, Bel-Venner MC (2010) Make love not war: when should less competitive males choose low-quality but defensible females? Am Nat 175(6):650–661
- Ward PI (1986) A comparative field study of the breeding behavior of a stream and a pond population of *Gammarus pulex* (Amphipoda). Oikos 46(1):29–36
- Watson PJ, Arnqvist G, Stallmann RR (1998) Sexual conflict and the energetic costs of mating and mate choice in water striders. Am Nat 151(1):46–58
- Wedell N, Kværnemo C, Lessells CKM, Tregenza T (2006) Sexual conflict and life histories. Anim Behav 71:999–1011
- Wong BBM, Candolin U (2005) How is female mate choice affected by male competition? Biol Rev Cam Phil Soc 80(4):559–571
- Zhu DH, Tanaka S (2002) Prolonged precopulatory mounting increases the length of copulation and sperm precedence in *Locusta migratoria* (Orthoptera: Acrididae). Ann Entomol Soc Am 95(3):370–373