



Selection of male traits during and after copulation in the seedbug *Lygaeus simulans* (Heteroptera, Lygaeidae)

ANDREAS TADLER*

Institut für Zoologie und Limnologie der Universität Innsbruck, Technikerstr. 25, A-6020 Innsbruck, Austria

HANS L. NEMESCHKAL AND GÜNTHER PASS

Institut für Zoologie der Universität Wien, Althanstr. 14, A-1090 Wien, Austria

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In order to evaluate selection of male morphological traits during copulation, a laboratory experiment was performed with the promiscuous seedbug *Lygaeus simulans*. Three male traits suspected as putative targets of selection were measured: weight, fluctuating asymmetry of a measure on the forewings, and length of a conspicuous genital structure, the processus gonopori. As fitness measures we considered total fecundity (number of fertilized eggs), insemination and fertilization success (established if a female laid fertilized eggs after copulation), and the interval between copulation and oviposition. Eighty-four males were allowed a single copulation with one virgin female each. Out of 67 copulations, 27 (40.2%) resulted in fertilized eggs and the oviposition latency ranged from 6 to 26 days. Regressions of male traits on the fitness measures showed significant phenotypical selection of two male traits: (1) males of average weight are more likely to achieve fertilization and (2) the oviposition latency was shorter for males with lower asymmetry. The copulation–oviposition interval may be especially important for male fertilization success because *Lygaeus* males perform copulatory mate guarding and the last male copulating with a female fertilizes most of the eggs. No selection of the genitalic trait was detected.

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ADDITIONAL KEY WORDS:—cryptic female choice – sexual selection – genitalia – reproduction – fertilization – insemination – sperm competition.

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* Corresponding author. E-mail: Andreas.Tadler@uibk.ac.at

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INTRODUCTION

Infertile copulations are common in various animal groups (review: Eberhard, 1996) and repeated mating may be a universal necessity for full fecundity and fertility in female insects (Ridley, 1988). This implies that an important opportunity for selection of males (variance in male fitness) arises during copulation. In particular, selection of males during copulation is one of the most important predictions of the cryptic female choice concept, which holds that females decide the fertilization success of males during or after genital coupling. Accordingly, the function of copulation is not only insemination but also to induce females to accept and use the sperm for egg fertilization (Eberhard, 1994, 1996). Hitherto, surprisingly few attempts have been made to evaluate whether this kind of selection exists at all (Eberhard, 1996).

Here we test whether actual phenotypic selection of male traits exists during or after copulation of the seedbug *Lygaeus simulans* Deckert 1985. In our approach, pairs of randomly assigned animals were allowed to copulate only once and relations between male phenotypic traits and several components of female reproductive output were evaluated. It is not possible with this design to disentangle natural and sexual selection and several mechanisms of selection acting only after female remating (e.g. sperm competition and discriminatory sperm usage by females) are excluded. However, cryptic female choice may take effect during or after single copulations because females may diminish the fertilization success of the first male in a variety of ways, e.g. by preventing complete intromission and insemination, reducing the number of offspring, or delaying offspring production (see Eberhard, 1996 for a broad discussion).

We investigated the existence of phenotypic selection of male traits by examining the relation between male characters and (1) number of fertilized eggs laid by the females, (2) fertilization success (yes or no), (3) insemination success (yes or no), and (4) the interval between copulation and oviposition. As the male characters actually selected were unknown, we chose three characters often discussed in context with sexual selection and female choice in the literature: male body weight, genital morphology and fluctuating asymmetry. Male weight and size is associated with copulatory success in a number of animals (Andersson, 1994). An important putative target for copulatory selection are genitalic structures (Eberhard, 1985; Arnqvist, 1997; Arnqvist, Thornhill & Rowe, 1997). As an example of an extravagant male genitalic character, we measured the length of a conspicuous extension of the aedeagus, the processus gonopori. The length of the processus shows an extreme variance within the family: in some species it is only a few hundred μm long whereas it exceeds the body length in several other species (Hopp, 1988). Fluctuating asymmetry is often associated with natural and sexual fitness (Møller & Swaddle, 1997). In our bugs we measured the asymmetry of the distance between two clearly defined marks on the forewings.

METHODS

A nomenclatural note

Lygaeus simulans Deckert, 1985 was not distinguished from its sibling species *L. equestris* (Linnaeus, 1758) prior to the taxonomic study of Deckert (1985). Both species share most of their distribution areas, often occur syntopically and almost no differences in their biology or behaviour are known (Deckert, 1985; Gusev & Tatarnikov, 1991). The species can be easily discriminated according to the diagnostic traits given in Deckert (1985) when a dissecting microscope is used, but clear discrimination is almost impossible with the naked eye. To date, there are no records of *L. simulans* from Scandinavia, so that Scandinavian works (e.g. Sillén-Tullberg, 1981) were most likely performed with *L. equestris*. However, it is not possible to relate works before 1985 unambiguously to the appropriate species name. We therefore refer to *L. equestris* s.l. (*sensu lato*) if it is uncertain whether *L. simulans* Deckert, *L. equestris* (Linnaeus), or a mixture of both species was investigated in previous studies.

Sexual biology of L. equestris s.l.

L. equestris s.l. females lay a series of egg clutches during their lives, with an interval of several days between each oviposition; they mate with one or several males before each oviposition event (Ludwig, 1926; Sillén-Tullberg, 1981). Oviposition sometimes takes place immediately after copulation (Ludwig, 1926), although the timing of oviposition is highly variable and influenced by day length, duration of hibernation and temperature (Solbreck, 1972; Solbreck & Sillén-Tullberg, 1981). Under long-day conditions, the animals first copulate about one week after adult eclosion. Egg maturation and oviposition is often induced by copulation; some females, however, already develop and lay mature eggs before copulation (Sillén-Tullberg, 1981). In the field the bugs copulate most often on their favourite host plant *Vincetoxicum hirundinaria*. There is no obvious role of long-distance attracting odours in the mate location or aggregation of sexually active bugs and no particular precopulatory courtship behaviour such as stridulation or display of male structures.

Sexual interaction can be roughly divided into two stages, namely a short precopulatory struggle and the copulation itself. During the precopulatory struggle the male mounts the female and grasps her with all his legs. As soon as both male and female face in the same direction, the male tries to tip up the female ovipositor with the aid of his parameres (movable external parts of the male genital capsule) and to insert his aedeagus into the female's bursa copulatrix. If this fails (in the present study about 30% of the mating attempts failed), the male gives up the attempt after a few minutes.

During copulation a stable conjunction between the external parts of genitalia (i.e. female ovipositor, male parameres and genital capsule) is established. The bugs thereby typically take on a tail-to-tail position in which they also move around and feed. In early copulation the male aedeagus performs a series of complicated movements and rotations inside the female bursa copulatrix. The aim of these manoeuvres is to insert the male processus gonopori into the female ductus receptaculi, an elastic tube connecting the female bursa copulatrix with the spermatheca (Ludwig, 1926; Bonhag & Wick, 1953). Sperm transfer failures are frequent, obviously most

often because the processus fails to enter the ductus (Micholitsch, 1997). There are no spermatophores; the filiform sperm move freely within the seminal fluid and within the receptaculum (Ludwig, 1926; see also Bonhag & Wick, 1953 for a detailed description of genitalia of Lygaeidae).

Copulation lasts from a few minutes up to 24 h (Ludwig, 1926). Ludwig (1926) interrupted copulation at different intervals and concluded that it takes at least 30 min from the beginning of copulation before sperm is transferred from the processus gonopori into the receptaculum. Sillén-Tullberg (1981) found that long-lasting (>15 h) copulations occur more frequently if the sex ratio is biased toward males or if gravid females are involved. Since prolonging copulation does not influence female fecundity or the P_2 value, she concluded that these prolonged copulations are a form of mate guarding and that the copulation duration is mainly determined by the male. It is, however, unclear whether the shorter copulations are terminated by the male or by the female. There is no postcopulatory mate guarding such as grasping or monitoring females after mating.

Origin of animals and housing conditions

The experimental animals descended from about 60 bugs collected on 7 and 22 September 1995 near Hundsheim, Lower Austria. In order to avoid pseudo-replication the laboratory-bred offspring of these wild captures were randomly assigned to pairs, and only one male and one female offspring from each of these pairs were used. The age of females (interval between adult eclosion and copulation) ranged from 15 to 33 days with a mean \pm SD of 25.0 ± 3.9 days ($n=83$).

Bugs were reared and bred under long-day conditions (18:6 h) at 28°C in plastic petri dishes which were provided with peeled sunflower seeds. Water was offered in vials with rolled rims (36 \times 11.5 mm) and four needle pricks in the plastic cover. All experimental animals were already isolated into separate petri dishes as last instars to ensure virginity.

Copulations and measurements

Eighty-four pairs of randomly assigned partners were used. Each animal had only a single opportunity to copulate and all copulations took place within one day. Partners were assigned at random and placed into separate vials. Interactions between animals were defined as copulation if a stable connection between their genitalia was established and the tail-to-tail position, typical for the copulation of Lygaeidae, was achieved. To ensure that each pair copulated only once, the animals were observed continuously and separated immediately after the release of genitalia. If no copulation took place within 4 h, animals were separated. As soon as copulations were finished, males were killed with CO₂ and stored in 80% alcohol. Females were monitored for oviposition every second day for the following 45 days and the number of eggs was counted (erroneous oviposition was also controlled on day seven). Since virgin females sometimes lay non-developing eggs, fertilization was considered a success only if eggs changed their colour from white to orange/red, indicating embryo development. Females that did not lay fertilized eggs after 45 days were killed and stored in 75% alcohol. These females were later dissected; whole mount

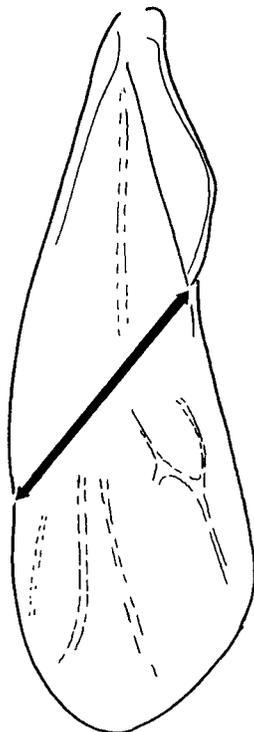


Figure 1. Forewing of *Lygaeus simulans*; the bar indicates the distance measured to assess asymmetry.

preparations were made from the receptacula and inspected for the presence of sperm using a light microscope (60×1.4 planapo DIC objective and interference contrast).

Only non-deformed males that achieved a copulation position were considered in the selection analysis. In three males the processus gonopori was completely deformed, two other males were excluded because of forewing deformations which probably occurred during ecdysis; a total of 67 pairs were thus available for subsequent analyses. Body weight of males was determined one day before copulation to the nearest 0.1 mg on an electrobalance; for statistical analysis we used the cube root of weight. Whole mount preparations were made of the male processus gonopori and the forewings (embedding medium Euparal). This allowed a spreading of the processus gonopori in two dimensions, avoiding the usual difficulties with measuring of three-dimensional structures. Measurements were taken under a microscope (magnification $40 \times$) with a drawing tube, a digitizing tablet with 1016 DPI resolution and the Sigma Scan program (Jandel Scientific). On the processus gonopori we measured the length from the middle of the "Übergangsstelle" (Ludwig, 1926, Abb. 8) to the tip. Asymmetry (absolute difference between left and right measures of an individual) was determined on the distance between two defined marks on the forewings (Fig. 1). This measure was chosen because it allows the most unambiguous definition of landmarks on this structure. The measurements were done by two persons, one of whom was not familiar with the design of the study or data from earlier measurements. Both observers measured the distance for each wing five times in succession. These five measurements were averaged

TABLE 1. Results of a two-way mixed-model ANOVA for testing the significance of the between-side variance of distances on the forewings. (Sides: fixed \times individuals: random, two measurements of each side.) *** $P < 0.001$

	SS	MS	df	F
Sides	2199.6	2199.6	1	1.56
Individuals	5817053.7	72713.1	80	51.59***
Interaction	112749.7	1409.3	80	5.51***
Error	41414.5	255.6	162	
Total	5973417.6	18493.6	323	

before any further analysis and calculation of asymmetry values. To test the significance of asymmetry values relative to measurement error we conducted a two-way mixed-model ANOVA with the left and right values of each observer as replicate measurements (Palmer & Strobeck, 1986; Palmer, 1994; Merilä & Björklund, 1995; Møller & Swaddle, 1997). Since the interaction side \times individual was significant, one can conclude that the between-side variation is significantly larger than the measurement error (Table 1). There was no evidence for directional asymmetry since the signed differences between left and right measures did not deviate significantly from zero (one-sample t -test, $n = 81$, $t = 25$, $P > 0.05$). The character did not demonstrate antisymmetry, as the distribution of signed character differences did not deviate significantly from a normal distribution (Kolmogorov–Smirnov goodness of fit test $n = 81$, $Z = 0.6505$, $P > 0.05$).

Analysis

In order to evaluate relations between male characters and fitness measures, we performed a series of univariate and multiple regressions. Multivariate regression reveals those characters that are more closely associated with the actual targets of selection within a set of characters (Lande & Arnold, 1983; Endler, 1986; Brodie, Moore & Janzen, 1995). In these regressions we used the standardized values of male forewing asymmetry, cube root of male weight, length of male processus gonopori and female age as independent variables. The univariate quadratic coefficients were estimated in models including the quadratic together with the linear terms (Endler, 1986). The linear multivariate coefficients were estimated in models including only the linear terms in order to exclude the influence of correlations between linear and quadratic terms (Brodie *et al.*, 1995). The multivariate quadratic coefficients were estimated in models including the linear, quadratic and, if possible, cross-product terms of male traits, together with the linear term of female age.

Several variables (the binary variables used as fitness measures, the number of eggs laid by females, male asymmetry) in these regressions did not meet the general assumption of normality of the conventional least squares technique. This may not affect the parameter estimates but could bias the significance tests of regression coefficients (Lande & Arnold, 1983). To overcome this, we assessed the statistical significance (P -values) of selection gradients using 10000 random permutations of the dependent variable per calculation, using the program RT (Manly, 1996). We chose this method because it is robust against any deviations from normality (Manly, 1996; Adams & Anthony, 1996) and it allows (unlike logistic regression) a direct estimation of the P -values of the selection gradients.

TABLE 2. Total fecundity analysis: univariate (β_1 , γ_1) and multivariate (β_2 , γ_2) selection coefficients (SE) of three male traits and female age. The fitness measure (number of fertile eggs/female) was zero if no eggs were laid or if eggs were infertile. Coefficient of determination of the multivariate model (including linear, quadratic and correlational terms of male traits) $r^2=0.276$, $n=67$, $P=0.0285$.
* $P<0.005/20$ ($=0.0025$, the critical P value after Bonferroni correction)

	β_1	P	β_2	P
Male forewing asymmetry	-0.22 (0.15)	0.1480	-0.28 (0.15)	0.0689
Cube root of male weight	-0.07 (0.15)	0.6570	-0.15 (0.15)	0.3492
Length of male processus	0.03 (0.15)	0.8552	-0.13 (0.16)	0.4182
Female age	-0.36 (0.14)	0.0151	-0.38 (0.14)	0.0094*
	$t\gamma_1$	P	γ_2	P
Male forewing asymmetry	-0.07 (0.15)	0.6403	-0.09 (0.14)	0.5578
Cube root of male weight	-0.25 (0.11)	0.0164	-0.42 (0.12)	0.0007*
Length of male processus	-0.05 (0.08)	0.5803	-0.08 (0.10)	0.4506
Female age	---	---	---	---

In a first step (total fecundity analysis, Table 2) we estimated the univariate and multivariate coefficients of selection during and after copulation. In this analysis we included all copulating pairs ($n=67$). The dependent variable (number of fertile eggs, zero if the female laid no eggs at all or only infertile eggs) was transformed according to the formula $y' = y^{(\gamma-1)} / ((\gamma * g)^{(\gamma-1)})$, where g is the geometric mean of the dependent variable. The constant γ was optimized using the routine BoxCox (StatSoft Inc.). The dependent variable was thereafter transformed into relative fitness (mean=1), so that the regression coefficients correspond to the selection coefficients in this analysis (Lande & Arnold, 1983). Because the coefficient of determination (r^2) of the multivariate model was significant, indicating that selection has actually occurred, a series of multiple regressions was performed in order to elucidate the mechanism of this selection. From these regressions we report the standardised partial regression coefficients; we made no attempt to transform them into selection coefficients.

The first question was whether selection was based on variance in fertilization success (one/zero) or on variance in the number of eggs within fertile copulations. To test this we performed two multiple regressions (fertilization and number of eggs analysis, Table 3). In the fertilization analysis we included all copulating pairs ($n=67$); a copulation was registered as successful (dependent variable=1) if the female laid fertilized eggs within 45 days after copulation, regardless of the number of eggs. The number of eggs analysis includes only the successful copulations ($n=27$). The dependent variable (number of eggs >0), was transformed according to the formula given above. Due the low number of successful pairs, we included into this analysis only the linear and quadratic terms of male characters together with female age as a linear term.

Two further multiple regressions (insemination and postinsemination analysis, Table 4) were conducted to test whether selection arose because of differences in insemination success or because of other reasons taking effect after insemination. In the insemination analysis we again included all copulating pairs ($n=67$). The dependent variable (insemination success) was set to one if sperm were found within the female receptaculum or if the female laid fertilized eggs; otherwise it was set to zero. The postinsemination

TABLE 3. Standardized partial regression coefficients of fertilization success and number of fertile eggs on three male characters and female age. The fertilization analysis includes all copulating pairs ($n=67$, model including linear, quadratic and correlational terms of male traits: $r^2=0.276$, $P=0.027$). In the number of eggs analysis, only fertilized batches were considered ($n=27$, model including linear and quadratic terms of male traits: $r^2=0.386$, $P=0.1676$)

	Fertilization (one/zero)			
	Linear	<i>P</i>	Quadratic	<i>P</i>
Male forewing asymmetry	-0.18	0.1482	-0.10	0.5194
Cube root of male weight	-0.15	0.2281	-0.41	0.0060
Length of male processus	0.09	0.5179	-0.08	0.6417
Female age	-0.34	0.0053	—	—
	Number of fertile eggs			
	Linear	<i>P</i>	Quadratic	<i>P</i>
Male forewing asymmetry	-0.43	0.0810	0.10	0.7101
Cube root of male weight	0.03	0.9162	-0.61	0.0175
Length of male processus	0.25	0.4503	0.06	0.7600
Female age	-0.22	0.3549	—	—

TABLE 4. Standardized partial regression coefficients of insemination and fertilization success on three male characters and female age. The insemination analysis included all copulating pairs ($n=67$, model including linear, quadratic and correlational terms of male traits: $r^2=0.232$, $P=0.089$). In the postinsemination analysis only pairs that achieved insemination success were included ($n=39$, model including linear, quadratic and correlational terms of male traits: $r^2=0.451$, $P=0.032$)

	Insemination (one/zero)			
	Linear	<i>P</i>	Quadratic	<i>P</i>
Male forewing asymmetry	-0.26	0.0351	-0.08	0.6160
Cube root of male weight	-0.17	0.1565	-0.18	0.2125
Length of male processus	0.19	0.1411	-0.07	0.6768
Female age	-0.36	0.0032	—	—
	Fertilization postinsemination (one/zero)			
	Linear	<i>P</i>	Quadratic	<i>P</i>
Male forewing asymmetry	-0.02	0.8949	-0.34	0.1175
Cube root of male weight	-0.10	0.6068	-0.73	0.0007
Length of male processus	-0.02	0.9305	-0.29	0.1447
Female age	-0.19	0.1297	—	—

analysis included only the successful pairs of the insemination analysis ($n=39$); the dependent variable was one if the female laid fertilized eggs. In a further step, we performed a multiple regression with the time between copulation and oviposition as a

TABLE 5. Standardized partial regression coefficients of interval between copulation and oviposition on three male traits and female age. Pairs were included into the analyses only if the female laid fertilized eggs ($n=27$, model including linear terms: $r^2=0.349$, $n=27$, $P=0.0440$. * $P<0.05/8=0.00625$, the critical P value after Bonferroni correction

	Linear	P	Quadratic	P
Male forewing asymmetry	0.66	0.0046*	-0.03	0.9061
Cube root of male weight	0.35	0.1753	-0.06	0.8149
Length of male processus	-0.21	0.4189	-0.16	0.4138
Female age	0.17	0.3985	—	—

dependent variable (Table 5). In this analysis, only fertile pairs were included ($n=27$). The low number of individuals barred including correlational trait values into this analysis.

The correlation coefficients within the males used for the regression analysis were significant between male weight and length of processus gonopori ($r=0.34$, $n=67$, $P<0.005$), and between forewing asymmetry and length of processus gonopori ($r=0.28$, $n=67$, $P<0.05$; note that this coefficient is not significant after adjusting the significance level for multiple comparisons). The correlation coefficient between asymmetry and male weight was not significant ($r=0.01$, $n=67$, $P>0.05$). Since none of the bivariate correlations was extremely high, no further test of multicollinearity was used.

RESULTS

Only 27 out of 67 copulations (40.3%) led to fertilized eggs. Within fertile females there was a considerable variance in the number of laid eggs (mean \pm SD 150.2 ± 91.7). Sixteen females laid infertile eggs. The number of eggs was considerably lower in these infertile females (mean \pm SD: 45.4 ± 49.2). The mean number (\pm SD) of egg-clutches/fertile female was 5.0 ± 3.1 , the mean number of clutches/infertile female was 2.1 ± 1.6 . The distribution of copula-duration with respect to insemination and fertilization success is shown in Figure 2. The shortest copulation leading to insemination was 71 min, the shortest leading to fertilization was 110 min. There was no significant relation between copula-duration and number of eggs, if only fertile copulations were considered (Spearman's $r = -0.04$, $P = 0.844$, $n = 27$).

The total fecundity analysis revealed a negative significant selection coefficient for the quadratic term of male weight and for the linear term of female age (Table 2). This indicates stabilizing selection of male weight and that older females were less fertile. The partial regression coefficients of the quadratic term of male weight were significant in the analysis of fertilization as well as in the number of eggs (Table 3), indicating that males with average weight not only achieved fertilizations more often, but also a higher number of eggs (only fertile copulations considered).

In 12 females that did not lay fertilized eggs, we found sperm within the receptacula, so that altogether 39 pairs did achieve insemination success. Although we did not attempt to quantify the number of sperm, there were obvious differences in sperm loading of receptacula. Four receptacula were filled nearly entirely with densely packed sperm strands, whereas spermatozoa were less numerous in other receptacula. The multiple regression of insemination success on the trait values

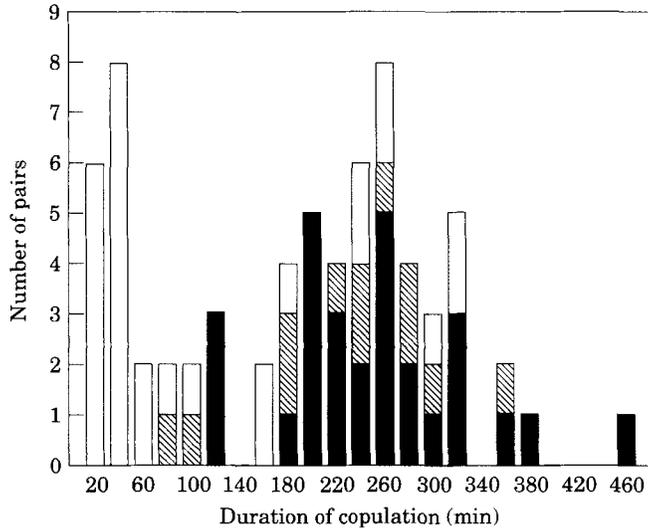


Figure 2. Distribution of durations of copulations without insemination (□), with insemination (▨) and with fertilization (■).

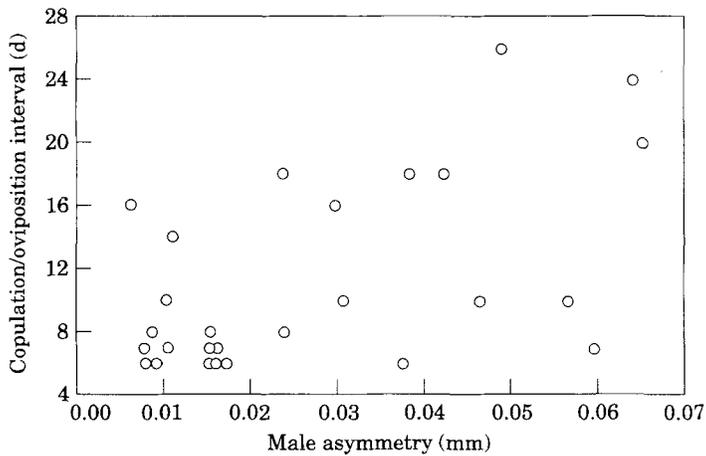


Figure 3. Bivariate plot of asymmetry of the forewing measure versus time between copulation and oviposition in days.

revealed a significant partial regression coefficient of female age. The quadratic regression coefficient of male weight was not significant, therefore the observed relation between male weight and fertilization success cannot be explained by differences in the abilities of males to achieve insemination. The quadratic coefficient of male weight was, however, significant in the postinsemination analysis, where only pairs that achieved insemination success were included (Table 4).

There was a high variability in the time between oviposition and copulation: some females began oviposition on the 6th day after copulation, the last female did so on the 26th day. The regression of days between copulation and oviposition on fluctuating asymmetry was significant in both the univariate and multivariate models (Table 5, Fig. 3).

DISCUSSION

The main finding of our study is that significant relations between male traits and components of female reproductive output in *L. simulans* already occur after the first copulation. We found stabilizing selection of male weight and a significant relation between male asymmetry and the interval between copulation and oviposition: this interval tended to be longer for more asymmetrical males. A key question is whether these relations are due to natural or sexual selection. Natural selection during copulation could involve (1) female-independent male malfunctions, (2) female malfunctions and (3) differences in the male's abilities to provide resources to the females (e.g. nutritional or defensive compounds of the ejaculate) (Eberhard, 1996).

Male malfunctions involve a male's inability to achieve successful fertilization (e.g. failure to produce an ejaculate, production of dead sperm, incapability of ejecting sperm into the female). If these malfunctions are completely independent of female properties and preferences, they constitute a form of natural selection (Eberhard, 1996). In our study copulations failed to result in fertilization for at least two reasons: insemination failures and fertilization failures despite insemination. The insemination and postinsemination analyses revealed that failures to produce sperm or to transfer sperm into the receptaculum were clearly not the reason for the stabilizing selection of male weight. Other forms of male malfunctions cannot be excluded. Female malfunctions or other kinds of female non-receptivity are a rather unlikely explanation for the observed selection pattern, since we assigned the partners at random and found significant relations between male traits and fertilization success. We did, however, find a relation between insemination success and female age: younger females were more likely to become inseminated. The reasons for this are unclear.

Another form of natural selection may occur if female fecundity depends on paternal resources provided by the male during copulation (Eberhard, 1996; Vahed, 1998). In *L. simulans*, no courtship feeding occurs and spermatophores or structures similar to the spermatophylax of *Ensifera* have never been observed. The only way in which the male could provide resources to the female would therefore be via the ejaculate. It is possible that male transfer substances inducing egg maturation and oviposition with the ejaculate and that these substances are responsible for the observed patterns of selection. However, the alternative explanation for the existence of such substances is that they are sexually selected and act not as a paternal investment, but as 'chemical genitalia' (Eberhard & Cordero, 1995).

At first glance one may argue for neglecting female choice altogether as an explanation for the observed selection patterns, because the selection was based on a considerable variance in female fitness. In our study, however, females were confronted for only a short time in their lives with a single male. This is an artificial situation unlikely to occur in *L. simulans* populations in the wild. If female choice is sequential the female may reduce her reproductive investment for a male with which she has already copulated, in favour of a future mate (Eberhard, 1996). This is feasible, if the costs of remating are very low for females (Dickinson, 1997). In *L. simulans*, costs of remating could be low or absent by several reasons. Females can feed during copulation and predation risks may be lowered because of the mutual reinforcement of the repellent effects of copulating animals on predators (see Gamberale & S. Tullberg, 1996 for the effectiveness of aposematic signals in a close relative of *Lygaeus*). Reducing egg numbers in favour of a future male may be also feasible because females spread their egg production over a period of several weeks

or months, and females copulate between ovipositions. The reduction of egg output after one copulation may be balanced by a higher output after another copulation. Furthermore, the bugs often occurs in high abundance (e.g. Ludwig, 1926; Solbreck, 1972), so that additional copulations will most often not be a limiting factor for female fitness. *L. equestris s.l.* provides a classical example for prolonged copulations as a form of mateguarding, i.e. males may prolong copulation beyond the time necessary for sperm transfer in order to prevent females from remating (Sillén-Tullberg, 1981). The present study revealed no relation between copulation duration and egg number, if only fertile copulations were considered. This result is in accordance with those of Sillén-Tullberg (1981), who reported that a postinsemination association is include in the copulation of *L. equestris s.l.* Copulatory mate guarding is only effective if the copulation–oviposition interval is short, so that the probability of female remating before oviposition can be effectively lowered by the prolonged copulation (Alcock, 1994). Male fitness will be therefore strongly influenced by the copulation–oviposition interval which gives the females the opportunity to influence the paternity of her offspring by altering the interval.

Sillén-Tullberg (1981) found that *L. equestris s.l.* females (enclosed in small boxes) are unable to reject the mating attempts of males, that copula duration is determined by males, and that the P_2 value is high. From this, one would expect male fertilization success to be exclusively a result of male competition. The present study shows such success cannot be attributed to male competition alone and suggests that females are able to bias male competition.

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REFERENCES

- Adams CA, Anthony CD. 1996.** Using randomisation techniques to analyse behavioural data. *Animal Behaviour* **51**: 773–738.
- Alcock J. 1994.** Postinsemination associations between males and females in insects: the mate-guarding hypothesis. *Annual Review of Entomology* **39**: 1–21.
- Andersson MB. 1994.** *Sexual Selection*. Princeton: Princeton University Press.
- Arnqvist G. 1997.** The evolution of animal genitalia: distinguishing between hypotheses by single species studies. *Biological Journal of the Linnean Society* **60**: 365–379.
- Arnqvist G, Thornhill R, Rowe L. 1997.** Evolution of animal genitalia: morphological correlates of fitness components in a water strider. *Journal of Evolutionary Biology* **10**: 613–640.
- Bonhag PF, Wick JR. 1953.** The functional anatomy of the male and female reproductive systems of the milkweed bug, *Oncopeltus fasciatus* (Dallas) (Heteroptera: Lygaeidae). *Journal of Morphology* **93**: 177–283.
- Brodie ED, Moore AJ, Janzen FJ. 1995.** Visualizing and quantifying natural selection. *Trends in Ecology and Evolution* **10**: 313–318.
- Deckert J. 1985.** Über *Lygaeus simulans* spec. nov. und *L. equestris* (Linnaeus 1758), zwei nahe verwandte

- paläarktische Lygaeinae (Heteroptera Lygaeidae). *Mitteilungen aus dem Zoologischen Museum in Berlin* **61**: 273–278.
- Dickinson JL. 1997.** Multiple mating, sperm competition and cryptic female choice in the leaf beetles (Coleoptera, Chrysomelidae). In: Choe JC, and Crespi J, eds. *The evolution of Mating Systems in Insects and Arachnids*. Cambridge: Cambridge University Press, 164–183.
- Eberhard WG. 1985.** *Sexual Selection and Animal Genitalia*. Cambridge, Mass.: Harvard University Press.
- Eberhard WG. 1994.** Evidence for widespread courtship during copulation in 131 species of insects and spiders, and implication for cryptic female choice. *Evolution* **48**: 711–733.
- Eberhard WG. 1996.** *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton: Princeton University Press.
- Eberhard WG, Cordero C. 1995.** Sexual selection by cryptic female choice on male seminal products – a new bridge between sexual selection and reproductive physiology. *Trends in Ecology & Evolution* **10**: 493–496.
- Endler JA. 1986.** *Natural Selection in the Wild*. Princeton: Princeton University Press.
- Gamberale G, S. Tullberg B. 1996.** Evidence for a more effective signal in aggregated aposematic prey. *Animal Behaviour* **52**: 597–601.
- Gusev RV, Tatarnikov DP. 1991.** On the distinguishing characters and distribution of the bugs *Lygaeus simulans* Deckert and *L. equestris* (Linnaeus) (Heteroptera Lygaeidae). [in Russian]. *Entomologicheskoye Obozrenie* **70**: 404–406.
- Hopp I. 1988.** Morphologisch-biologische Untersuchungen an Genitalstrukturen, Flügeln und Vorderbeinen der Lygaeiden (Heteroptera) – Ein Beitrag zur Problematik der Erstellung eines phylogenetischen Systems. Unpublished Dr Phil. Thesis, Universität Freiburg.
- Lande R, Arnold SJ. 1983.** The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Ludwig W. 1926.** Untersuchungen über den Copulationsapparat der Baumwanzen. *Zeitschrift für Morphologie und Ökologie der Tiere* **5**: 291–380.
- Manly BFJ. 1996.** *Randomisation and Monte Carlo Methods in Biology* 2nd Ed. London: Chapman & Hall.
- Merilä J, Björklund M. 1995.** Fluctuating asymmetry and measurement error. *Systematical Biology* **44**: 97–101.
- Micholitsch T. 1997.** Insemination and fertilization in *Lygaeus simulans* (Heteroptera: Lygaeidae): are there indications for “cryptic female choice”? Mag. Thesis, Universität Wien.
- Møller AP, Swaddle JP. 1997.** *Asymmetry, Developmental Stability, and Evolution*. Oxford: Oxford University Press.
- Palmer AR, Strobeck C. 1986.** Fluctuating asymmetry: measurement, analysis, patterns. *Annual Review of Ecology and Systematics* **17**: 391–421.
- Palmer AR. 1994.** Fluctuating asymmetry analyses: a primer. In: Markow TA, ed. *Developmental Instability: Its Origins and Evolutionary Implications*. Dordrecht: Kluwer Academic Publishers, 335–364.
- Ridley M. 1988.** Mating frequency and fecundity in insects. *Biological Reviews* **63**: 509–549.
- Sillén-Tullberg B. 1981.** Prolonged copulation: a male postcopulatory strategy in a promiscuous species, *Lygaeus equestris* (Heteroptera: Lygaeidae). *Behavioral Ecology and Sociobiology* **9**: 283–289.
- Solbreck C. 1972.** Sexual cycle, and changes in feeding activity and fat body size in relation to migration in *Lygaeus equestris* (L.) (Het., Lygaeidae). *Entomologia Scandinavia* **3**: 267–274.
- Solbreck C, Sillén-Tullberg B. 1981.** Control of diapause in a “monovoltine” insect, *Lygaeus equestris* (Heteroptera). *Oikos* **36**: 68–74.
- Vahed K. 1998.** The function of nuptial feeding in insects: a review of empirical studies. *Biological Review* **73**: 43–78.