Artificial selection and heritability of sperm length in *Gryllus bimaculatus*

EDWARD H. MORROW* & MATTHEW J. G. GAGE†

Population and Evolutionary Biology Research Group, Nicholson Building, School of Biological Sciences, University of Liverpool, Liverpool, L69 3BX, U.K.

The adaptive significance of variation in sperm size remains poorly understood but there has been even less attention focused on the genetic mechanisms controlling spermatozoal traits (only three species have been studied). Here we explore heritability and artificial selection of sperm length in the cricket *Gryllus bimaculatus*. Using a within-family selection design we selected sperm with increasing and decreasing sperm size and bred divergence across five generations. Although we recorded no significant parent–offspring heritability, realized heritability from a within-family analysis was significant at 0.52 ± 0.06 SE ($P = 0.01$) and we successfully achieved significant divergence. However, we only achieved a response when the maternal line was incorporated into selection (from the F_1 onwards) and our findings therefore suggest that sperm length in *G. bimaculatus* is a sex-linked trait that is influenced by genes which are active on the female chromosome. Accordingly, sperm length heritability in *G. bimaculatus* can only be measured using a within-family design because the spermatozoal phenotype is sex-limited (to males) while the genotype is sex-linked (to females). The evolutionary significance of the heritability of sperm length is discussed with reference to sex-linkage of this important sex-limited trait.

Keywords: artificial selection, *Gryllus bimaculatus*, heritability, sperm competition, sperm length.

Introduction

Spermatozoal size and structure show great variation across species (Stockley *et al.*, 1997; Birkhead & Møller, 1998; Gage *et al.*, 1998; Morrow & Gage, 2000) from the tiny aflagellate amoeboid gametes of nematodes (LaMunyon & Ward, 1998) to the giant sperm of Drosophilids (Pitnick *et al.*, 1995). Reasons for this variation in sperm structure remain poorly understood, but recently studies have attempted to explain why there is variation in sperm size. In internal fertilisers, sperm have evolved to function within the environment of the female reproductive tract and sperm size is associated with female reproductive tract dimensions across ptiled beetles (Dybas & Dybas, 1981), birds (Briskie & Montgomerie, 1992), Drosophilids (Pitnick *et al.*, 1995, 1999), diopsid flies (Presgraves *et al.*, 1999) and moths (Morrow & Gage, 2000). Sperm competition (when sperm from different males mix and compete for a female's ova, Parker, 1970) is one force that is positively

(birds: Briskie & Montgomerie, 1992; butterflies: Gage, 1994; primates and rodents: Gomendio & Roldan, 1991) and negatively (fishes: Stockley *et al.*, 1997) associated with sperm size across taxa. There is also evidence from single-species studies that increased sperm size improves a male's fertilization success in mites (Radwan, 1996), nematodes (LaMunyon & Ward, 1998) and dung flies (Otronen *et al.*, 1997).

In addition to between-species differences, there is significant variation in sperm size between individual males within a species. Between-male variation in sperm head size was first identified in bulls (Williams & Savage, 1925) and humans (Moench & Holt, 1932). Subsequently, significant intraspecific variation in total sperm length has been found for several species of rodents (Friend, 1936; Braden, 1959; Illisson, 1969), insects (Otronen *et al.*, 1997; Ward, 1998), and other taxa (Ward, 1998). Ward (1998) predicted that this intra-specific variation in sperm morphometry could be widespread and Morrow & Gage (2001) screened 10 species from a wide range of invertebrate and vertebrate taxa using identical techniques and found that significant between-male variation in sperm size was recorded for every species measured. The study included examinations of populations that were likely to have low

*Correspondence and present address: Department of Ecology and Environmental Science, University of Umeå, SE 901-87 Umeå, Sweden. E-mail: Ted.Morrow@eg.umu.se

†Present address: School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K.

heterozygosity, such as laboratory or island populations. In addition to total sperm length, there was also universal and significant between-male variance in the sizes of the head, midpiece and flagellum in every species in which these components were visible (Morrow & Gage, 2001). The evolutionary reasons for persistent differences between males in sperm morphometry are still unclear, as are the consequences to the fertility or fertilization success of individual males. Sperm size is unlikely to be a neutral trait given the well documented interspecific evidence (see Birkhead & Møller, 1998) and given that sperm represent a significant stage in male reproduction, and therefore are the focus of intense selection.

Despite attempting to explain the adaptive significance of variation in sperm size, there is limited understanding of the genetic mechanisms controlling variation in sperm size. Sperm size is not likely to be a condition-dependent trait since all males within a population are selected for competition and fertilization within a common reproductive environment. The few studies which have explored condition-dependence in sperm size concur with this interpretation (Vawda & Mandlwana, 1990; Blay & Yuval, 1997; Birkhead *et al.*, 1999), for example, male moths raised on a severely limiting diet partitioned resources away from sperm number production but maintained sperm size (Gage & Cook, 1994). Estimates of heritability (h^2) are available for several sperm traits from rabbits (*Oryctolagus cuniculus* sperm head length 0.72 ± 0.18 ; width 0.71 ± 0.13 ; area 0.74 ± 0.15 ; and shape 0.56 ± 0.13 (Napier, 1961)), mice (*Mus musculus* midpiece length 0.97 ± 0.36 (Woolley & Beatty, 1967) and 0.76 ± 0.02 (Woolley, 1971); and head shape ≈ 0.9 (Illis, 1969)) and dung flies (*Scathophaga stercoraria* sperm length ≈ 0.67 (Ward, 2000)). All of these estimates are high relative to those found for other fitness traits (mean $h^2 \pm \text{SE}$ life history 0.26 ± 0.01 ; physiology 0.33 ± 0.03 ; behaviour 0.30 ± 0.02 ; morphology 0.46 ± 0.004 (Mousseau & Roff, 1987)).

In this study we use the natural variation in sperm size between males as the basis for artificial selection upon sperm length in order to establish the degree of response to selection. Selection experiments on the midpiece length of mouse sperm showed strong responses to selection and high estimates of heritability, from 0.76 ± 0.02 to 0.97 ± 0.36 (Woolley & Beatty, 1967; Woolley, 1971) but only 13 generations were followed. Here we use the field cricket *Gryllus bimaculatus* as our genetic model. Not only does sperm size vary significantly between males of the same population, but we know that sperm length is male-specific and is consistent for individual males producing spermatophores on different days (Morrow & Gage, 2001).

In addition to examining responses to selection, we explore whether sperm length genes are active in the male or female genome. Ward (2000) has shown sperm length to be heritable in the dung fly and that genes coding for sperm length may well be X-linked; thus selection on males and females may be important to the evolution of sperm length. *Gryllus bimaculatus*, in common with the majority of the Orthoptera, has an XO/XX system of sex determination (Hewitt, 1979). Thus sex-linkage of sperm size in this species, if it occurred, would be restricted to the X chromosome. We therefore explore the heritability of sperm length in *G. bimaculatus* using a within-family artificial selection design (Falconer & Mackay, 1996) that allows us both to measure the degree of genetic response, and to quantify any sex-linkage.

Materials and methods

Study animal and rearing conditions

The cricket *Gryllus bimaculatus* (Gryllidae) is a hemimetabolous exopterygote that can be reared under laboratory conditions. In order to minimize any possible environmental effects on sperm size, all crickets at every stage of their life cycle and for each generation were reared under identical conditions. Individuals were reared at $28 \pm 2^\circ\text{C}$ under a 14:10 L:D light regime. Adults and nymphs were provided with food *ad libitum* in the form of rodent pellets (CRM (P), Special Diet Services, U.K.) and water (in vials plugged with cotton wool). Mating pairs were provided with an oviposition dish (replaced on alternate days) comprising a 50-mm diameter Petri dish base filled with a wad of damp cotton wool, into which females will readily oviposit. Removed dishes were kept damp and covered with a lightly fitting lid and incubated at $28 \pm 2^\circ\text{C}$ under a 14:10 L:D cycle until larval crickets began to emerge approximately 10 days later.

Spermatophore collection and sperm measurement

Males were selected according to the mean length of sperm sampled from a spermatophore collected at least 10 days after adult eclosion, when sexually mature (Simmons, 1986).

Male *G. bimaculatus* produce a single spermatophore at a time, and it hardens within 1–2 h after which it may be gently removed from within the valves surrounding the genital opening using a pair of soft forceps. Spermatophores removed in this way were submerged in 20 mL of distilled water and cracked open using a pair of modified pincer-like forceps. The sperm were

gently dispersed by agitating the sperm mass and spermatophore fragments with a pair of straight forceps for approximately 30 s. This resulted in an even dispersion of the entire ejaculate without sperm clumping and with a negligible frequency of fragmented sperm. Severe sperm clumping occurs if spermatophores are allowed to evacuate normally into water.

A direct method was used to measure sperm total length. Magnified phase-contrast images of sperm were relayed by video to a flat-screen monitor and the image then drawn onto an acetate sheet. These tracings were photocopied onto paper and measurements of their length taken using a Silva online-5 digital map-measurer (Field & Trek Ltd, U.K.). This method of measuring sperm length is highly repeatable both between measuring events (repeated measures GLM, $F = 155\ 033$, $P < 0.001$) and between observers (repeated measures GLM, $F = 184\ 710$, $P < 0.001$) and has been used successfully in other studies (e.g. Morrow & Gage, 2000; Morrow & Gage, 2001). Mean sperm length was determined for each male from measures of 10 intact sperm and this level of sampling results in a representative mean (Morrow & Gage, 2000). Mean sperm length in *G. bimaculatus* measured in this way has been found to range from less than 900 μm to over 1000 μm (Morrow & Gage, 2001).

Artificial selection protocol

Initially 39 virgin male crickets were removed randomly from the stock population to be included in the founding population. Each individual was isolated prior to pairing in a small cage (0.5 L) and provided with *ad libitum* food and water. All 39 males were screened for sperm length, and the 10 longest and 10 shortest males were used to establish 'long' and 'short' familial lines. In this first P generation, 'long' or 'short' males were paired with random stock virgin females.

The 20 separate families of larval crickets were reared to maturity under the same environmental conditions as the stock, but in 5-L cages. In this F_1 generation, five males from each of the 'long' and 'short' families were screened for sperm length ($n = 100$ males in total). Within each family, the male with either the longest or shortest sperm was then chosen to be the sire of the next generation. Accordingly, 10 'long' and 10 'short' families were continued and this regime was repeated and carried forward to the F_4 generation.

In the F_1 and subsequent generations, the female 'long' or 'short' genotype was incorporated into the selection protocol. Virgin females were randomly isolated from each family and paired with males from the same selection criteria (i.e. either 'long' or 'short') but not from the same family within the sperm length line.

In subsequent generations the families from which the females were taken were rotated around the 10 families of the 'long' or 'short' lines to minimize inbreeding.

Initial estimate of heritability

To measure the response to selection an initial estimate of heritability must be determined. It must be noted that if there is sex-linkage of a trait then this value can be an under-estimate because the offspring phenotype may not be under the control of the paternal genotype. Offspring-parent regressions were determined over two generations before selection had begun. Because sperm lengths were measured for offspring from either end of the parental distribution (leading to bimodality), our initial estimate of heritability was based on a regression using a bimodal distribution. The offspring means were weighted because of unequal sample sizes within families (mean n of offspring = 4.85, range = 4–5; Kempthorne & Tandon, 1953; page 179, Falconer & Mackay, 1996). The weighting factor w_n is applied to the mean values of n offspring from each family. The estimate of heritability (h^2) is simply double the coefficient of the slope (b_{Op}), because only one parent's phenotype is measured. Thus $h^2 = 2b_{Op}$.

Heritability and response using within-family selection

A strict within-family design was employed as the method of selection. This enables both calculation of the degree of response, and also shows whether sex-linkage occurs. The realized heritability (h_w^2) is therefore calculated from a modified version of that calculated for an individual selection design (h^2) (p. 236, Falconer & Mackay, 1996):

$$h_w^2 = h \frac{(1 - r)}{(1 - t)}$$

In addition the calculation for the expected response may also be modified to include the additional information provided by knowledge of individuals measured in the context of their respective families (p. 236, Falconer & Mackay, 1996).

Realized heritability

The response to selection also provides an estimate of the realized heritability from the regression of response over the cumulative selection differential. As there were no control line measurements available, the response was calculated as generation means for each line subtracted from one another. The cumulative selection differential must also be appropriately weighted accord-

ingly because the number of offspring measured within each family was not maintained at exactly five. The selection differentials from each line are added to one another for each round of selection (Falconer & Mackay, 1996).

Results

Initial estimate of heritability

Because of unequal numbers of full-sib offspring measured per family we applied a weighting correction to the heritability estimate (p. 179 Falconer & Mackay, 1996). Sperm length heritability was 0.29 (± 0.20) which was not significant ($y = 0.15x + 841$, $F = 2.24$, $r^2 = 0.11$, $P = 0.15$, $n = 20$) but it produces an initial estimate that can be used for the primary aim of measuring responses to selection (Falconer & Mackay, 1996). It should be noted that this nonsignificant result could be explained if there was a sex-linked effect from the female genotype which, for sperm, obviously cannot be phenotypically described in a simple parent-offspring relationship.

Response to selection

Estimates for the average responses per generation from F_1 to F_4 are presented in Fig. 1 and by generation in Table 1. Responses were $r^2 = 0.95$, $P = 0.03$ and $r^2 = 0.66$, $P = 0.18$ for the 'short' and 'long' lines, respectively (short $y = -6.56x + 982.9$; long $y = 5.59x + 983.5$; Fig. 1).

The mean divergence per generation is presented in Fig. 2 ($y = 12.15x + 0.63$) and by generation in

Fig. 1 Response of the short line (closed symbol) and the long line (open symbol) over four generations of selection for sperm length in *Gryllus bimaculatus* (generation means are given in μm). Note that offspring traits from the P generation return to the population mean and a response is only achieved from F_1 onwards when the maternal genotype is incorporated into selection.

Table 1 Expected responses (in μm) calculated for comparison with the actual response observed to selection for sperm length in *Gryllus bimaculatus*

Line	Expected response when $h^2 = 0.29$	Observed response from F_1 to F_2
Short	4.52	11.28
Long	4.24	9.09

Fig. 2 Divergence between short and long lines over four generations (F_1 – F_4) of selection for sperm length in *Gryllus bimaculatus* (generation means are given in μm).

Table 2 Estimates of average response (in μm) per generation (R) to selection for sperm length in *Gryllus bimaculatus*

	Mean response
Short	6.56 ± 1.12
Long	5.59 ± 2.81
Divergence	12.15 ± 3.40

Table 2. Although 86% of the variation was explained by the regression line ($r^2 = 0.86$, $P = 0.07$), only four rounds of selection generates no statistical significance, despite an apparent response.

Heritability and response using within-family selection

The within-family estimate of heritability (h_w^2) was 0.1956. The within-family response R_w calculated for the short line was 2.34, and for the long line was 2.19.

The expected response of within-family selection may be compared with the expected response of individual selection by dividing one by the other, thus:

Fig. 3 Response vs. selection for the two selection lines of *Gryllus bimaculatus* combined, providing an estimate of realized heritability ($h^2 \pm \text{SE}$) from the slope of the regression of 0.52 ± 0.06 .

$$R_w/R = (1 - r)\sqrt{\{(n - 1)/[n(1 - t)]\}} = 0.52$$

where $n = 5$ offspring and t is the intraclass correlation coefficient, estimated from the offspring–parent regression. In other words the efficiency of the within-family selection relative to an alternative individual selection protocol is approximately one half.

Estimate of realized heritability

The primary aim of this study was to estimate realized heritability in order to measure the degree of response to selection. Realized heritability, calculated from the slope of a line fitted to the combined generation means vs. the cumulative selection differentials, was 0.52 ± 0.06 ($r^2 = 0.98$, $F = 79.33$, $n = 4$, $P = 0.01$; Fig. 3). The slopes for the upward (long) and downward (short) selected lines were 0.30 ± 0.02 ($r^2 = 0.97$, $F = 72.02$, $n = 4$, $P = 0.01$) and 0.41 ± 0.05 ($r^2 = 0.98$, $F = 17.45$, $n = 4$, $P = 0.053$), respectively.

Discussion

Our selection experiment shows that sperm length in *Gryllus bimaculatus* does have a considerable genetic component; elongation and reduction of sperm length was successfully achieved using a within-family design of artificial selection (Falconer & Mackay, 1996) and realized heritability was significant with $h^2 = 0.52$. Selection was attained over a relatively short period involving four rounds of within-family selection proper.

Our experiment was not designed to provide a reliable estimate of heritability from an individual parent–offspring regression, because we also anticipated that sperm length could be under genetic control from the

maternal genotype. Heritability of single-sex traits such as gamete phenotype cannot be fully measured using offspring–parent regression because the phenotype is only expressed in a single parent. Furthermore, if the expressed trait is genotypically coded by the non-expressing parent, it is impossible to measure parent–offspring heritability. This phenomenon may explain why we calculated a nonsignificant estimate of h^2 for sperm length from the offspring–parent regression (0.29), but a significant realized heritability from the within-family estimate (0.52) when maternal genotypes were incorporated into the selection protocol from F_1 to F_4 (Figs 1–3), although genetic drift cannot be ruled out.

Heritability estimates of sperm dimensions have been measured for only three other species: rabbits, mice and yellow dung flies (see Introduction). Dung flies (*Scathophaga stercoraria*) have significantly heritable sperm length at 0.67 (Ward, 2000), however, Ward also showed that sperm length within the dung fly may be sex-linked to the X-chromosome because there was a significant relationship between a male's sperm length and his maternal grandfather (Ward, 2000). In our study of *G. bimaculatus* we find similar evidence that sperm length may be influenced by female-specific genes. The sex determination system within *G. bimaculatus* is XO/XX (Hewitt, 1979) and males therefore only have one set of sex chromosomes, in contrast to dung flies that have an additional Y-chromosome. We have yet to determine the nature of sex-linkage for sperm-length genes and whether linkage is partial or complete. Sex-linkage of genes may only be estimated using information on trait size from reciprocal hybrid experiments, where the trait values of these hybrids are compared to the difference between the parental lines used to create them (Reinhold, 1998).

Traditionally, traits that are closely related to fitness are expected to have lower heritabilities because of stabilizing selection (Fisher, 1930; Falconer & Mackay, 1996). This is supported by an analysis of a large number of traits by Mousseau & Roff (1987) who found that life history traits generally had lower heritabilities than morphological traits. We do not yet know the degree to which sperm length is subject to stabilizing or directional selection, or both. Sperm competition could select directionally and there is evidence that sperm competition level and success are associated with bigger sperm (LaMunyon & Ward, 1998, 1999). However the female reproductive tract is an environment likely to generate stabilizing selection and there is evidence for associations between storage site dimensions and sperm size across taxa (e.g. Briskie & Montgomerie, 1992; Pitnick *et al.*, 1999; Presgraves *et al.*, 1999; Morrow & Gage, 2000). Whatever the forces selecting for sperm length optima, it is counterintuitive that a male fitness

trait is controlled by genes that are active on maternal chromosomes. If this is the mechanism for sperm phenotype control, then it does explain why we record significant variation between individual males within a population in sperm size. In a survey of 10 vertebrate and invertebrate taxa, Morrow & Gage (2001) found significant variation in sperm head, midpiece, flagellum and total dimensions between males within a population. If sperm size is maintained through indirect selection (since the females carrying the genes cannot themselves be selected) then between-male variation in sperm size could be maintained. It remains unclear, however, why a seemingly important fitness trait such as the male gamete is controlled by genes that cannot be selected directly.

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