Inheritance of Male Parental Investment in an Insect

Scott K. Sakaluk, Robert L. Smith

American Naturalist, Volume 132, Issue 4 (Oct., 1988), 594-601.

Vol. 132, No. 4 The American Naturalist October 1988

NOTES AND COMMENTS

INHERITANCE OF MALE PARENTAL INVESTMENT IN AN INSECT

Courtship feeding of females by males and other forms of male parental investment (Trivers 1972) are common throughout the animal kingdom (see Thornhill 1976; Ridley 1978; Smith 1979; Zeh and Smith 1985; Quinn and Sakaluk 1986). It has never been shown empirically, however, that differences between males in their willingness to invest in offspring or mates have a genetic basis. In the decorated cricket, *Gryllodes supplicans* (Orthoptera: Gryllidae), a male provisions his mate with a gelatinous spermatophylax, a specialized portion of the sperm-transfer vessel (spermatophore), which the female eats after mating (Sakaluk 1984, 1987). The size of the spermatophylax is an important male fitness trait, influencing the degree to which a female permits herself to be inseminated (Sakaluk 1985). Evolutionary theory predicts that such discrimination by females should deplete the additive genetic variance underlying traits important to a male's reproductive success (Williams 1975; Maynard Smith 1978; Thornhill 1980). In contrast to this prediction, we now report a significant heritability of the relative investment by males in their spermatophores, specifically, that portion of the spermatophore constituting a parental investment.

In crickets, mating is completed when a male successfully transfers a spermatophore, which remains attached outside the female's body. The spermatophore of a *G. supplicans* male consists of a large spermatophylax, devoid of sperm, which surrounds a smaller sperm-containing ampulla. A *G. supplicans* female detaches the spermatophylax from the sperm ampulla within a few seconds of copulation and feeds on it for about 40 min. Because it takes longer to eat a larger spermatophylax and because the ampulla is removed minutes after the spermatophylax is consumed, a female penalizes a male that provides a smaller nuptial meal by removing his ampulla before it is emptied of sperm (Sakaluk 1984, 1985, 1987). Premature removal of the ampulla may result in the transfer of sperm insufficient to fertilize all of a female's eggs (Sakaluk 1984), or it may reduce the competitiveness of a male's ejaculate in a numerical sperm competition (Sakaluk 1986*a*).

METHODS OF STUDY

Crickets were collected as late-instar nymphs at various locations in Tucson, Arizona. Newly eclosed adults were housed individually in uniquely labeled,

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plastic shoe boxes and provisioned with ample food (dry dog food coated with honey and salad oil), water, and shelter. Cages were kept in an environmental chamber maintained at 31°C ± 2° and a relative humidity of 20%–40% and exposed to the ambient photoperiod. Each male and the two components of his spermatophore were weighed using a Mettler H54AR analytical balance accurate to 0.01 mg. Spermatophores were obtained by gently pulling on the spermatophylax portion visible in the male's spermatophoric pouch (Alexander and Otte 1967). The spermatophylax emerged intact and separated cleanly from the sperm ampulla, which was also removed and weighed. Spermatophores were air-dried for 24 h, then dried to constant weight in a drying oven for 0.5 h at 58°C just before a second weighing. We obtained data on either the first or second spermatophore ever produced by each male, measured at 10–12 days of adult age ($N = 55$). To obtain some indication of the consistency of spermatophore size over time, another spermatophore was obtained 6.96 ± 0.95 days later (mean ± SE; range, 1–17 days) from 23 of these males. A virgin female was subsequently introduced into each male's cage, along with a 0.1-liter weigh boat filled with moistened sand to serve as an oviposition substrate. Thirty-one pairs produced offspring, which were used in the heritability study.

We reared sons of known sires and determined heritabilities of quantitative traits known or suspected to be important in male mating success. Fifty to 60 offspring of each male were reared in 19-liter buckets provisioned with ample food (a mixture of chicken feed and dry cat food), water, and shelter and held in a rearing room maintained at 29°C ± 3° on a photoperiod of 16 h light and 8 h dark. Buckets were repositioned within the rearing room several times per week to reduce environmental effects. Male offspring were transferred to the environmental chamber after their final molt, maintained in the same way as their sires, and measured at the same adult age. Family sizes were unequal across traits because of missing values for some sires.

RESULTS

Despite its obvious importance to a male's fertilization success, spermatophore mass varied widely, ranging from 3.7 mg to 13.5 mg (mean, 8.2 ± 0.2 SE) in 55 field-collected males. There was a positive correlation between the mass of the first (or second) spermatophore ever produced by a male and the mass of another of his spermatophores measured 1–17 days later ($r = 0.47$, $P < 0.025$). The spermatophylax accounted for 83% (72%–89%) of the mass of the spermatophore compared to 17% (11%–28%) for the sperm ampulla (determined for first or second spermatophore only). A phenotypic correlation matrix revealed interesting differences in the investment by males in the two components (table 1). The mass of the spermatophylax correlated positively with male body mass, and the proportions of body mass contributed to the spermatophylax by large and small males were similar. In contrast, the mass of the sperm ampulla was relatively constant across body size, with larger males devoting proportionally less of their body weight to the ampulla. Thus, males of varying body mass delivered ejacu-

TABLE 1

PHENOTYPIC CORRELATION MATRIX FOR MALE DECORATED CRICKETS

Trait	SM	SM/BM	PM	PM/BM	AM	AM/PM	DSM	DPM	DAM*
Body mass (BM)	0.568	0.006†	0.579	0.026†	0.144†	−0.550	0.614	0.638	0.205†
Spermatophore mass (SM)		0.820	0.992	0.807	0.326	−0.132†	0.919	0.903	0.351
Investment in spermatophore (SM/BM)			0.756	0.982	0.258†	0.255†	0.704	0.593	0.234†
Spermatophylax mass (PM)				0.825	0.216†	−0.219†	0.842	0.891	0.248†
Investment in spermatophylax (PM/BM)					0.137†	0.081†	0.597	0.646	0.125†
Ampulla mass (AM)						0.739	0.531	0.328	0.894
Investment in ampulla (AM/PM)							0.028†	−0.169†	0.618
Dry spermatophore mass (DSM)								0.966	0.583
Dry spermatophylax mass (DPM)									0.355

* DAM, dry ampulla mass.
† Not significant.

lates of similar magnitude but provisioned females with nuptial meals that differed greatly in size.

The narrow-sense heritability of each trait (h^2) was determined as twice the regression of male offspring on sires, and its standard error was obtained by doubling the standard error of the regression (table 2; Falconer 1981). Because unequal numbers of sons were measured for each sire, the analysis was performed using offspring means weighted by family size and the intraclass correlation coefficient (Falconer 1963). The offspring-on-sire regression is one of the most conservative estimates of heritability because it avoids the covariance arising from maternal effects, dominance variance, and environmental effects (Falconer 1981).

Male body mass and the absolute mass of the spermatophore and its component parts were not significantly heritable (table 2). However, the proportion of body mass contributed to the spermatophore was characterized by a relatively high and statistically significant heritability. The heritability of relative spermatophore investment was due primarily to the proportion of male body mass devoted to the

TABLE 2

HERITABILITIES OF MALE BODY SIZE AND SPERMATOPHORE INVESTMENT IN CRICKETS

Trait	h^2	SE	P*	Families	Offspring
Body mass (BM)	−0.034	0.208	NS	29	229
Spermatophore mass (SM)	0.236	0.195	NS	31	250
Investment in spermatophore (SM/BM)	0.486	0.202	<0.025	29	250
Spermatophylax mass (PM)	0.199	0.196	NS	29	239
Investment in spermatophylax (PM/BM)	0.468	0.205	<0.05	27	217
Ampulla mass (AM)	0.255	0.206	NS	29	239
Investment in ampulla (AM/PM)	0.202	0.175	NS	27	216
Dry spermatophore mass (DSM)	−0.097	0.220	NS	31	248
Dry spermatophylax mass (DPM)	−0.063	0.318	NS	29	239
Dry ampulla mass (DAM)	0.079	0.222	NS	29	237

* NS, not significant.

spermatophylax. Relative spermatophylax investment was significantly heritable; the proportion of body mass contributed to the ampulla was not.

DISCUSSION

Several factors impinge on the heritability estimates reported here. First, laboratory studies of this sort generally tend to minimize environmental variation and thus increase the probability of detecting additive genetic variance. In this study, however, sires were obtained as late-instar nymphs from the field, and data were limited to F_1 offspring. Because of the greater environmental variance present in sires, these results probably underestimate additive genetic variance (Zeh 1987). Another concern is possible paternal effects arising through nutrients contained in the spermatophylax. Large spermatophylaxes produced by certain offspring could be the result of their mothers' being fed larger and presumably more-nutritious spermatophylaxes: such an effect would tend to inflate the father-son regression. We believe that a paternally derived nutritional link is unlikely because females, as well as their mates and offspring, were maintained on a high-protein diet and food was provided ad libitum. Under these conditions, nutrients obtained through the consumption of spermatophores should not appreciably augment the investment by females in their eggs. Moreover, because the observed son-sire resemblance is in the relative rather than absolute size of the spermatophylax, the link cannot be a nutritional effect of the absolute size of the sire's spermatophylax (Gwynne, pers. comm.).

In other insects, particularly in the Lepidoptera, spermatophore size decreases

over additional matings (Boggs 1981; Rutowski 1984; Svärd 1985). We restricted our measurements to the first or second spermatophore ever produced by a *G. supplicans* male in order to avoid the variance introduced by a mating-number effect. This precaution may have been unnecessary because the mass of spermatophores produced by *G. supplicans* males does not differ significantly from those produced by the same males 24 h later (Sakaluk 1985). In the present study we found a positive correlation between the mass of a male's first (or second) spermatophore and that of one measured several days later. Taken together, these results indicate that *G. supplicans* males hold constant their investment in spermatophores across additional matings.

Accurate estimates of genetic correlations require much larger sample sizes than those reported here (Falconer 1981). Nonetheless, the difference in heritability of the proportion of body mass contributed to the spermatophylax versus that contributed to ampulla, along with the lack of a phenotypic correlation between the two traits, suggests that investments in spermatophore components are genetically uncoupled. Investment in the ampulla undoubtedly reflects the number of sperm required to fertilize a female's eggs, taking into account the numerical sperm competition that occurs when a female mates with other males (Sakaluk 1986*a*). Investment in the spermatophylax, however, is probably determined by a more complex array of trade-offs involving the degree to which females are inseminated (Sakaluk 1984), the time and energy required to manufacture larger spermatophylaxes (Sakaluk 1985), and potential fitness benefits derived through the incorporation of spermatophore nutrients into eggs fertilized by the male (Friedel and Gillott 1977; Boggs and Gilbert 1979; Gwynne 1984*a*; Butlin et al. 1987).

The fate of nutrients contained in the spermatophylax has not been investigated for *G. supplicans*. Studies of other insects, however, have demonstrated the incorporation of spermatophore nutrients into a female's developing oocytes (Friedel and Gillott 1977; Boggs and Gilbert 1979; Butlin et al. 1987), and spermatophylax consumption in another ensiferan, *Requena verticalis* (Orthoptera: Tettigoniidae), has been shown to effect an increase in both the size and number of eggs produced (Gwynne 1984*a*). Theft of spermatophylaxes from mated *G. supplicans* females by nearby conspecifics, and the concomitant struggles to retain courtship food gifts, provides evidence of a nutritional benefit for this species (Sakaluk 1987). Thus, the spermatophylax can be viewed as a male parental investment, assuming that it contributes to offspring fathered by the investing male. This latter assumption is met in *G. supplicans* because of the use of mixed sperm by females mated to more than one male (Sakaluk 1986*a*,*b*).

The factors maintaining heritability of relative spermatophylax investment are unknown. It seems clear that body size, which is itself environmentally determined, places an upper limit on the size of a spermatophore that a male can produce, presumably because of some physical or energetic constraint. For each size class, however, there must exist a range of possible spermatophore investments from which a male may "choose": a male could opt to produce the largest spermatophore permitted by his size class, becoming a high-investment (HI) male,

or invest a smaller proportion of his body mass, becoming a low-investment (LI) male.

One way in which the genetic variation underlying relative spermatophylax investment could be maintained is if the net benefits to HI and LI males fluctuated according to food availability (or varied geographically; Gwynne 1984*b*). In a favorable environment, both HI and LI crickets would generally attain large body sizes. In such a situation, LI males would probably be at an advantage because reduced investment in courtship gifts would permit more-frequent mating; yet spermatophylaxes would still be large enough to ensure adequate insemination of females (see Sakaluk 1984, 1985). In contrast, HI males would copulate less frequently, and nutritional benefits to females would be minimized in an environment in which food was not limiting. In an unfavorable environment, crickets would generally develop into smaller adults and the selective balance would be reversed. Spermatophylaxes produced by LI males would be so small that females would occasionally remove sperm ampullae prematurely, placing such males at a competitive disadvantage (Sakaluk 1985). In contrast, spermatophylaxes provided by HI males would still accomplish complete sperm transfer, and the fitness benefits obtained by females through spermatophore consumption would be greater under these conditions.

Various mechanisms such as mutation, immigration, trade-offs among fitness components, fluctuating selection pressures, and disruptive selection have been proposed for the maintenance of genetic variation of traits important to male mating success (Lande 1976; Cade 1981, 1984; Hamilton and Zuk 1982; Gross 1985). A possible factor maintaining heritability of relative spermatophylax investment is that the ability of males to inseminate their mates fully (and the nutritional benefits derived by females) fluctuates according to food availability. Alternatively, the Arizona study population may have been founded by migrants from more than one population, each exhibiting a pattern of spermatophore investment uniquely suited to its own environment. Thus, the additive genetic variance detected in the Arizona population could be a consequence merely of a genetically heterogeneous founding population (R. D. Alexander, pers. comm.).

Fluctuating selection, in the form of acoustically orienting parasitoid flies, has previously been invoked to explain the high heritability of calling duration in male field crickets (Cade 1981). Additional studies have demonstrated significant heritable variation in other traits crucial to male sexual performance and reproduction (Cade 1984), including those related to female choice (Simmons 1987). We now add courtship food gifts to the expanding list of important male fitness traits characterized by significant additive genetic variance.

ACKNOWLEDGMENTS

This study was supported by a postdoctoral fellowship from the Natural Sciences and Engineering Research Council of Canada (S.K.S.) and an Arizona Agricultural Experiment Station Project grant (R.L.S.). We thank R. D. Alexander, C. L. Boggs, D. N. Byrne, D. T. Gwynne, N. A. Moran, M. C. Sakaluk,

F. G. Werner, D. W. Zeh, J. A. Zeh, and two anonymous reviewers for helpful comments on the manuscript, and M. C. Sakaluk for valuable field and laboratory assistance.

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SCOTT K. SAKALUK*
ROBERT L. SMITH

DEPARTMENT OF ENTOMOLOGY
UNIVERSITY OF ARIZONA
TUCSON, ARIZONA 85721

Submitted September 18, 1987; Accepted February 5, 1988

* Present address: Ecology Group, Department of Biological Sciences, Illinois State University, Normal, Illinois 61761.