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Quantitative genetics of seminal receptacle length in *Drosophila melanogaster*

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The length of the female's primary sperm-storage organ, the seminal receptacle, has undergone rapid divergence within the *Drosophila* genus. Quantitative genetic analysis of seminal receptacle length was carried out on two laboratory strains of *Drosophila melanogaster* that had undergone artificial selection for both increased and decreased organ length. Realized heritabilities were 0.176 and 0.270 for the two experiments. Parental strains, F_1, F_{1r} (reciprocal), F_2, backcross, and backcross reciprocal generations were used in a line-cross (generation means) analysis. This analysis revealed that additive, dominance, and additive-by-dominance epistasis contributed significantly to the means. No significant maternal effects were found. Variance analysis indicated that a completely additive model was adequate to explain the variances observed in these lines. Castle–Wright minimal estimates of 5.25 and 1.91, segregating loci responsible for mean differences, were found for the two respective experiments. There were significant positive correlations between additive effects of seminal receptacle length and thorax length in both experiments. The correlated evolution of sperm and seminal receptacle length is discussed.

Keywords: artificial selection, female choice, heritability, line crosses, means analysis, sperm.

Introduction

Sperm length has diverged dramatically in the genus *Drosophila*, with total length varying from 0.23 mm in *D. subobscura* (Snook, 1997) to over 58 mm in *D. bifurca* (Pitnick *et al*., 1995b). Equally variable is the length of one of the female's sperm-storage organs, the seminal receptacle (SR), which ranges from 0.41 mm to 81.67 mm (Pitnick *et al*., 1999), with the longest receptacles being over 20× the length of the female's body. Although females also possess a pair of spermathecae, the SR appears to be the primary source of sperm for fertilization in most *Drosophila* species (Pitnick *et al*., 1999). While controlling for phylogenetic effects, Pitnick *et al*. (1999) compared 46 species throughout the genus and found that SR length is highly correlated with sperm length ($r^2 = 0.900$, $P < 0.001$). Similar relationships have been identified in comparative studies of stalk-eyed flies (Presgraves *et al*., 1999), featherwinged beetles (Dybas & Dybas, 1981), and birds (Briskie & Montgomerie, 1993), as well as between sperm length and the length of the spermathecal duct in moths (Morrow & Gage, 2000).

Examination of geographical variation in sperm and SR length throughout the range of the Sonoran Desert endemic fly, *D. mojavensis*, has revealed significant among-population differences in both traits in addition to a pattern of strong correlated evolution between these male and female traits (S. Pitnick, G. T. Miller & T. A. Markow, unpubl. data). This finding is consistent with speculation that rapid coevolution of sperm and seminal receptacle length provides a widespread source of reproductive isolation (Pitnick *et al*., 1999). Specifically, *D. mojavensis* is believed to be a species in a state of incipient speciation (Markow & Hocutt, 1998), and these data reveal that sperm and SR length are evolving at a sufficiently rapid pace to be relevant to the speciation process.

Correlated evolution of sperm and female sperm-storage organ length has also been postulated to result from postcopulatory sexual selection, with length of the SR serving as the proximate mechanism underlying female sperm choice (Eberhard, 1996; Pitnick *et al*., 1999). It is hypothesized that there is morphological compatibility between sperm and SR such that, for example, within relatively long SRs, relatively long sperm have an advantage in the competition to fertilize ova (Pitnick *et al*., 1999). Analysis of SR length presents a unique opportunity to study the genetics of putative female choice on an easily quantifiable trait without the problems inherent in the genetic analysis of behavioural traits (Arnold, 1994).

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Although the role of SR length in speciation and sexual selection is still highly speculative, the rapidly divergent nature of this trait and central role of this organ in reproduction (i.e. sperm storage and egg fertilization) makes it a worthwhile subject for genetic analysis. Here we report a quantitative genetic analysis of SR length in *Drosophila melanogaster*. Bidirectional artificial selection for SR length was performed in order to create distinct lines for measuring heritability. Means analysis was then performed, using line crosses on the end products of selection, to model the contributions of additive, dominance, epistatic, and maternal genetic contributions. Finally, variance analysis was performed to assess these models and to estimate the number of genetic elements that determined the selected difference between the high and low lines. Correlated responses in sperm length in the selection lines and the results of experiments using the selection lines to determine the adaptive significance of variation in SR length, will be reported elsewhere.

Materials and methods

Culturing

The experimental population was a strain of *D. melanogaster* founded in 1996 from 50 isofemale lines collected from a Napa Valley, CA, USA, vineyard. The lines were combined and maintained continuously in a large population cage supporting at least 1000 individuals on standard cornmeal–molasses–agar medium. Fifteen virgin male/female pairs were sampled from this population cage to establish the first SR selection experiment in February 1998. A second selection experiment was performed on a strain of *D. melanogaster* with a sepia eye mutation (se #1668 obtained from the Bloomington Stock Center), after backcrossing four times into the Napa Valley strain. Fifteen male/female pairs were sampled to establish the second experiment in July 1998. Sepia flies were used in the second experiment to facilitate paternity designation in future sperm competition experiments after selection was complete.

Selection protocol

For each experiment, the 15 virgin pairs were each placed in 8-mL food vials for 2 days and then discarded. Larval density was uncontrolled during selection; each vial contained an estimated 40–60 developing larvae. From each of the 15 vials, 5 male and 5 female offspring were collected within eight hours of eclosion. At four days of age, 75 female and 75 male flies were paired randomly, avoiding sib matings. Two days later, the males were discarded and the females were dissected to determine SR length. The vials that contained the females with the 15 smallest and the 15 largest SR lengths were used to establish the low and high lines, respectively. Selection continued unabated for 23 and 13 generations in the first and second experiments, respectively. Thereafter, gains realized by selection were maintained by selecting at the same level every other generation until genetic analysis was performed in generation 39 for the first experiment and generation 26 for the second experiment.

SR length was determined for each female as follows. Following anaesthetization with ether, the reproductive tract was dissected into phosphate-buffered saline (PBS) on a microscope slide. A glass coverslip was placed on top with clay at the corners that allowed flattening of the SR to two dimensions without stretching the organ. The preparation was then viewed at 200× with an Olympus BX60 microscope with Nemarsky optics. A digitized image of the SR was captured using a Dage CCD72 camera and organ length determined using the public domain NIH Image program.

The final round of selection for SR length resulted in a high line (H) and a low line (L) for each experiment. Each pair of H and L lines was used separately to generate 9 lines, of which 4 were nonsegregating lines (parental H and L, F_1 and F_{1r}) and 5 segregating lines (F_2 and 4 backcrosses). Sources of these lines are detailed in Table 1. Rearing conditions were standardized for all lines by transferring 50 first instar larvae to each 8-dram vial containing 8 mL of medium. This density approximates the conditions observed throughout the course of selection. The means and variances of SR length and thorax length of these lines were used to analyse the quantitative genetics by generation means or line cross analysis (Mather & Jinks, 1982; Lynch & Walsh, 1998). Line variances were used to evaluate the adequacy of an additive model and to estimate the effective number of genes (Lynch & Walsh, 1998) responsible for the difference between the end points of selection. The methods for the means and variance analyses are outlined below.

Means analysis

Two genetic models based on the 9 lines were evaluated using each experiment separately. The first model assumed both composite additive [d] and dominance [h] effects due to nuclear genes in the line and maternal composite additive [d_m] and dominance [h_m] effects due to nuclear genes in the mother of each line. Note that in these crosses a maternal cytoplasmic effect is not distinguishable from a maternal nuclear composite additive effect.

Table 1 Source of genetic lines used in the generation means and variance analyses

Line	Source		
	Designation	Female	Male
High	H	H	H
Backcross High	BH	H	F_{1p}[a]
Backcross High reciprocal	BH_r	F_{1p}	H
First filial generation	F_1	H	L
First filial generation reciprocal	F_{1r}	L	H
Second filial generation	F_2	F_{1p}	F_{1p}
Backcross Low reciprocal	BL_r	F_{1p}	L
Backcross Low	BL	L	F_{1p}
Low	L	L	L

[a] F_{1p} is equal numbers of F_1 and F_{1r} pooled together.

The second model was based only on composite nuclear gene effects in each line; additive [d], dominance [h] and three digenic epistatic effects: additive by additive [i], additive by dominance [j] and dominance by dominance [l]. Because the genetic effects of the reciprocal crosses (e.g. BL and BL_r) are identical in this model the reciprocals were pooled with their corresponding line cross.

For all models, weighted least-squares procedures were used to estimate the parameters contained in vector **y** and their variances from the diagonal of their variance covariance matrix **S** (Mather & Jinks, 1982; Lynch & Walsh, 1998). The estimates of **y** and **S** are obtained as:

$$\hat{\mathbf{y}} = (\mathbf{C}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{C})^{-1}\mathbf{C}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{x}$$

and

$$\hat{\mathbf{S}} = (\mathbf{C}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{C})^{-1},$$

where **C** is the coefficient matrix for the contribution of effects to each line mean, **V** is the diagonal matrix of the error variances of each line mean, and **x** is the vector of observed line means. Goodness of fit of each model was tested using a χ^2, where

$$\chi^2 = \mathbf{x}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{x} \quad \mathbf{x}^{\mathrm{T}}\mathbf{V}^{-1}\mathbf{C}\hat{\mathbf{y}} \quad \text{(Hayman, 1958)}.$$

The degrees of freedom for this χ^2 is the number of line means minus the number of parameters in the model. Significance of model parameters were evaluated using *F*-statistics by comparing improvement of the goodness of fit after modifying model parameters.

Genetic correlation between SR length and thorax length was calculated using a technique of resampling lines. Resampling ($r = 1000$ replicates) was performed by sampling randomly with replacement the observations of each line until n_i (n_i = observed number of i^{th} line) samples were obtained. The vector of resampled line means **xr** were then used to estimate the genetic parameters in vector **y** for the best model identified in the means analysis for each trait as:

$$\hat{\mathbf{y}} = (\mathbf{C}^{\mathrm{T}}\mathbf{C})^{-1}\mathbf{C}^{\mathrm{T}}\mathbf{xr}.$$

From the r estimate of **y**, the ordered statistics provide the confidence interval of the composite genetic effects (i.e. the 95% confidence interval is bounded by order resample estimates # 25 and # 975).

The genetic correlations for model effects of the SR and thorax length traits were calculated from the paired estimates of each resample. The matrix of these correlation coefficients thus estimates the genetic effects correlation matrix between SR and thorax length.

Variance analysis

Line variances were analysed by the method outlined by Lynch & Walsh (1998, pp. 226–231). In our case, we reduced the vector of variance components to parental and segregational variance, as suggested by Lynch and Walsh, and used the iterative process of Hayman (1960) to calculate maximum-likelihood estimates of the variances. Goodness-of-fit was determined by a χ^2 that compared observed variances with the maximum-likelihood estimates of those variances in the additive model.

The variance estimates from the above procedures were used to find Castle–Wright estimators (Lynch & Walsh, 1998) of the minimum number of segregating loci responsible for the difference in means of the high and low line for each experiment.

Results

Direct response to selection

The response to selection for both increased and decreased SR length as a function of both generation

and cumulative selection differential is illustrated in Fig. 1. The regressions of combined high and low line cumulative response on cumulative selection differential are highly significant ($P < 0.0001$) for both experiments. Because the SR is a female-specific organ, the regressions estimate one half of the realized heritability of the trait (Falconer & Mackay, 1996). The realized heritabilities are 0.176 for the first experiment and 0.270 for the second experiment.

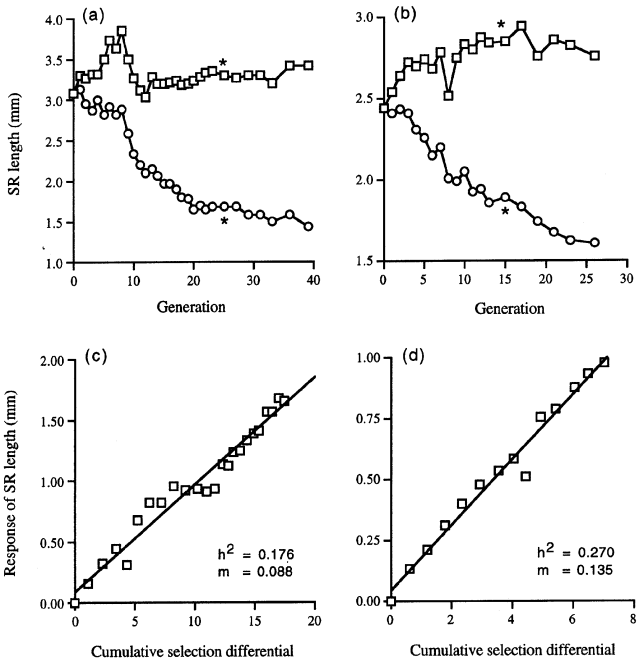

Fig. 1 Response in (a) the first and (b) the second high (squares) and low (circles) SR selection lines, and the response in (c) the first and (d) the second experiments as a function of cumulative selection differential. The heritability is twice the slope (m) of the regression. Asterisks indicate the first generation of relaxed selection (see text for details).

The distributions of SR length and thorax length for each experiment and each line were tested for normality with the Shapiro–Wilk Statistic, W. All of the SR length distributions were normally distributed ($0.95 > P > 0.06$), while two of the 18 thorax length distributions were significantly different ($P < 0.05$) from normality. These two distributions were both from replicate one and involved backcrosses to the low line. Their deviation from normality was caused by a negative skewness that was not serious enough to require transformation. Table 2 lists the means, variances and sample sizes for each line, experiment and trait.

Means analysis

Table 3 lists the results of the generation means analysis for both experiments and both traits. Experiment 1 did not have any significant maternal effects for either trait. The nuclear model showed that additive, dominance and additive-by-dominance epistasis contributed significantly to the means. A model with only these effects was adequate ($\chi^2_2 = 5.87$, $P = 0.05$). In the case of thorax length, only additive and dominance effects were significant with no apparent epistatic influences. The non-epistatic model with m, [d] and [h] was adequate to describe the line means ($\chi^2_3 = 2.25$, $P = 0.52$).

Experiment 2 was similar to experiment 1. There were no significant maternal effects. The thorax length trait was explained by [d] and [h] ($\chi^2_3 = 4.78$, $P = 0.19$), as in experiment 1. SR length did not require dominance or epistatic parameters to explain adequately the generation means, as a simple additive model was sufficient ($\chi^2_4 = 2.52$, $P = 0.64$).

Resampling of the data to estimate the parameters and their correlations between traits was carried out

Table 2 Means, variances, and sample sizes for both SR and thorax length for nine line crosses of each experiment

	Experiment 1						Experiment 2					
	SR			TL*			SR			TL*		
Line	Mean	Var	*n*	Mean	Var	*n*	Mean	Var	*n*	Mean	Var	*n*
H	3.149	0.059	15	80.20	3.74	15	2.561	0.027	15	77.07	3.50	15
BH	2.593	0.115	30	79.17	6.76	30	2.320	0.092	30	77.67	9.75	30
BH_r	2.623	0.075	30	79.37	3.00	30	2.378	0.110	30	76.27	13.93	30
F_1	2.150	0.032	20	79.90	2.94	20	2.067	0.021	20	76.80	3.85	20
F_{1r}	1.999	0.068	20	78.40	3.10	20	2.035	0.070	20	76.35	5.29	20
F_2	2.290	0.143	50	78.46	13.72	50	2.103	0.108	50	76.78	8.99	50
BL_r	1.822	0.047	30	76.33	11.47	30	1.821	0.047	30	75.00	10.28	30
BL	1.866	0.062	30	78.57	5.50	30	1.790	0.070	30	74.23	12.39	30
L	1.360	0.012	15	74.53	11.55	15	1.499	0.019	15	72.87	3.84	15

*Thorax lengths are reported in micrometer units (80 units = 1 mm).

Table 3 Estimates of parameters for maternal and nuclear gene models. Values indicate contribution to phenotype of both Seminal Receptacle (SR) and Thorax length (TL). Bold numbers indicate significant contribution for that parameter ($\alpha = 0.05$)

	Maternal		Nuclear		Best model	
	exp 1	exp 2	exp 1	exp 2	exp 1	exp 2
SR						
m	2.252	2.033	2.511	2.132	2.292	2.060
[d]	**0.837**	**0.529**	**0.895**	**0.531**	**0.920**	**0.536**
[h]	**−0.132**	0.027	−0.446	−0.035	**−0.174**	
[dm]	0.028	0.005				
[hm]	0.057	0.051				
[i]		−0.256	−0.102			
[j]		−0.261	0.024	**−0.334**		
[l]		0.009	−0.046			
χ^2	13.46	0.39	—	—	5.87	2.52
d.f.	4	4	—	—	2	4
TL						
m	78.03	75.02	77.77	78.92	77.56	75.15
[d]	**1.84**	**1.79**	**2.83**	**2.10**	**2.31**	**2.16**
[h]	**1.25**	**1.58**	1.37	−6.21	**1.55**	**1.60**
[dm]	0.25	0.40				
[hm]	−0.40	0.44				
[i]		−0.41	−3.95			
[j]		−2.03	0.50			
[l]		0.01	3.87			
χ^2	17.55	5.14	—	—	2.25	4.78
d.f.	4	4	—	—	3	3

Table 4 Genetic effects correlation matrix for genetic parameters of the best generation means model for Seminal Receptacle (SR) length and Thorax length (TL). Each experiment is analysed separately

		Exp 1			**Exp 2**
		SR			SR
		[d]	[h]	[j]	[d]
TL	[d]	0.123**	0.098**	−0.010	0.110**
	[h]	0.120**	0.182**	−0.088*	−0.020*

**$P < 0.001$, *$P < 0.05$.

with the best models (Table 3) of each experiment. The genetic effects correlation matrix for each experiment (Table 4) shows significant positive correlations between additive effects of SR length and thorax length for both experiments.

Variance analysis

Variance analysis showed that an additive model was adequate to explain the differences in variances for the lines produced in the crosses for SR length but less adequate for thorax length. Table 5 lists the estimates of parental and segregational variance and the goodness-of-fit statistic for both experiments and both traits. The Castle–Wright estimators of the number of segregating loci (minimal estimate) are also listed in Table 5.

Discussion

Response by SR length to artificial selection

As one might expect from the rapid evolutionary divergence observed in SR length throughout the genus *Drosophila* (Hihara & Kurokawa, 1987; Joly & Bressac, 1994; Pitnick *et al*., 1999), selection was successful in both directions. The response to selection appears to be asymmetrical in both experiments, with the response to selection for low SR length greater than the response in the upward direction. Heritabilities were different for the two experiments, with the value derived from the second experiment substantially higher.

Each of these disparities may be attributable to measurement error; SR length is an exceptionally difficult trait to measure accurately. The measurement of SR length requires the receptacle to be flattened into two dimensions, and any overcompression can artificially increase the measurement. The first experiment began several generations prior to the second, and measurement error was greatly reduced as we became more

	SR		TL	
	Rep 1	Rep 2	Rep 1	Rep 2
Var (H)	0.082 (0.015)	0.056 (0.012)	2.656 (0.831)	4.493 (1.227)
Var (L)	0.012 (0.004)	0.020 (0.006)	7.598 (1.578)	4.503 (1.228)
Var (S)	0.070 (0.018)	0.074 (0.017)	5.810 (1.862)	10.294 (2.189)
χ^2_3 (P)	3.46 (0.33)	4.41 (0.22)	10.18 (0.02)	7.57 (0.06)
n_e	5.25 (1.42)	1.91 (0.48)	0.67 (0.32)	0.38 (0.13)

Table 5 Variance analysis estimates for parental (H, L) and segregational (S) variation, and minimum effective number (*ne*) of genes responsible for the differences in the means of the High and Low lines for both Seminal Receptacle (SR) length and Thorax length (TL). SE in parentheses

proficient. This error was attributable specifically to variation in the volume of saline present between the slide and the coverslip. Because the accuracy with which we were able to measure SR length was far superior at the end of selection than earlier during the experiment, we re-measured the base populations during the same generation that data for the quantitative analyses were collected. SR length (mean ± SE) for each base population was more central than illustrated in Fig. 1 (experiment 1: 2.280 ± 0.038: experiment 2: 2.145 ± 0.027). These results are consistent with the response of selection being relatively symmetrical. The realized heritability of experiment 1 from generation 10 onward, is 0.25, very close to what was realized in experiment 2. These values are also very close to the value which Mousseau & Roff (1987) reported for the mean heritability of life-history traits that have a history of strong selection acting upon them. It should be noted that the high measurement error (especially for the earlier generations) for this trait makes our heritability values minimum estimates.

The lack of a limit to selection response after 13 generations of selection in experiment 2 and 23 generations in experiment 1 seems surprising considering the variance estimate of only five genetic elements, in experiment 1, and only two genetic elements, in experiment 2, contributing to trait divergence. Differences between experiments in the number of contributing genes were probably the result of experiment 2 having more loci fixed at the start of selection. The sepia line (a severely inbred laboratory stock) was backcrossed into the Napa Valley line flies four times prior to selection. This may not have been sufficient to generate variation at all contributing loci.

In general, the rate of response to selection by a trait is limited only by the amount of additive genetic variation and by trade-offs with other fitness traits undergoing simultaneous selection (Lande, 1979; Falconer & Mackay, 1996). Strong directional selection will tend to deplete the additive genetic variance and lower the ability of a population to increase its mean fitness (Fisher, 1958). Several mechanisms that serve to maintain additive genetic variation in the face of selection have been proposed (reviewed in Roff, 1992; Stearns, 1992). Traits subject to strong sexual selection, as has been postulated for SR length, would seem to be especially vulnerable to loss of additive variance because of the persistent directional selection necessary for their evolution.

SR length was not limited by low V_a

The large response to artificial selection for SR length in both directions indicates substantial additive genetic variation remaining in the trait. No evidence of a decline in V_a or heritability was detected over the course of selection. Also, the low heritabilities observed were not indicative of a lack of ability to respond to selection. The rate of natural selection depends on the amount of additive genetic variance available, and not necessarily on the heritability (Houle, 1992). As long as there is sufficient V_a, high levels of epistasis or dominance should not encumber traits undergoing rapid evolution. For the current analysis, a completely additive model was able to explain the variance observed in both experiments and nonadditive variation made only minor contributions to the observed trait. The low heritabilities are thus presumed to be the result of high environmental variance (some of which may have been a consequence of the inherent measurement error).

No evidence for trade-offs limiting response of SR length

Limits on the evolution of life-history traits in large natural populations are probably not imposed by the exhaustion of genetic variation for any one trait, but by trade-offs among traits with significant phenotypic and genetic covariances (Stearns, 1992). Directional selection will generally then be halted by these trade-offs and result in a stabilizing selection that will deplete V_a. If true, then traits that are able to respond rapidly to selection over long periods of time are predicted to have fewer negative genetic correlations with other life-history traits. Such limits to rapid evolution may best be illustrated by the widely observed phenomenon of artificially selected traits that

quickly return to their original means after selection is relaxed (Falconer & Mackay, 1996). When selection for SR length was relaxed to every other generation, no regression in the response was detected as would be expected if there was a large cost involved (Stearns, 1992; Falconer & Mackay, 1996). In fact, following completion of this study and five generations without selection, SR length divergence remained unchanged (Figs 1a, 1b).

Correlated evolution of SR and sperm length and sex-specific costs

The limited cost of increasing SR length (i.e. the putative female preference trait) is what is generally expected in sexual selection models, where the high cost of the exaggerated male trait (i.e. longer sperm) is assumed to limit the response to selection (Andersson, 1994). Substantive costs to males associated with the production of relatively long sperm have been identified, including delayed reproductive maturation, dedication of a greater portion of reproductive effort to spermatogenesis, and the production and transfer of relatively few sperm (Pitnick *et al*., 1995a; Pitnick, 1996). With respect to females, a positive genetic correlation between SR length and body size was found in the current study (Table 4; cf. TL for high and low lines in Table 2). Whereas in *Drosophila* body size positively correlates with many measures of fitness (Tantawy, 1961; Partridge & Farquhar, 1983; Mackay, 1985), it is also known to be positively correlated with delayed larval development time, which can be costly (Hillesheim & Stearns, 1991; Zwaan *et al*., 1995). Moreover, the low SR lines consistently exhibit more rapid development and greater longevity relative to the high SR lines, independent of body size differences (Miller and Pitnick, unpubl. data). These potential fitness costs of increased SR length may limit the evolution of this trait in natural populations of *Drosophila*. However, our experimental protocol largely eliminated selection on development time and longevity in these lines. These important fitness trade-offs therefore would not have limited the selection response or resulted in regression of SR length following relaxation of selection in the laboratory.

In summary, SR length is a relatively simple trait with seemingly few genetic elements determining large differences in length. Few nonadditive effects are evident. There is evidence from comparative studies for rapid coevolution of SR length and sperm length (Pitnick *et al*., 1999; unpubl. data); costs of increasing the length of these traits may be imposed upon both males and females. The role of SR length in generating selection on sperm size requires further exploration.

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References

ANDERSSON, M. 1994. *Sexual Selection*. Princeton University Press, Princeton, NJ.

ARNOLD, S. J. 1994. Multivariate inheritance and evolution: A review of concepts. In: Boake, C. R. B. (ed.) *Quantitative Genetic Studies of Behavioral Evolution*, pp. 17–49. The University of Chicago Press, Chicago, IL.

BRISKIE, J. V. AND MONTGOMERIE, R. 1993. Patterns of sperm storage in relation to sperm competition in passerine birds. *Condor*, **95**, 442–454.

DYBAS, L. K. AND DYBAS, H. S. 1981. Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. *Evolution*, **35**, 168–174.

EBERHARD, W. G. 1996. *Female Control: Sexual Selection by Cryptic Female Choice*. Princeton University Press, Princeton, NJ.

FALCONER, D. S. AND MACKAY, T. F. C. 1996. *Introduction to Quantitative Genetics*, 4th edn. Longman, Essex.

FISHER, R. A. 1958. *The Genetical Theory of Natural Selection*. Dover Publications, Inc., New York.

HAYMAN, B. I. 1958. The separation of epistatic from additive and dominance variation in generation means. *Heredity*, **12**, 371–390.

HAYMAN, B. I. 1960. Maximum likelihood estimation of genetic components of variation. *Biometrics*, **16**, 369–381.

HIHARA, F. AND KUROKAWA, H. 1987. The sperm length and the internal reproductive organs of *Drosophila* with special references to phylogenetic relationships. *Zool. Sci*., **4**, 167–174.

HILLESHEIM, E. AND STEARNS, S. C. 1991. The responses of *Drosophila melanogaster* to artificial selection on body weight and its phenotypc plasticity in two larval food environments. *Evolution*, **45**, 1901–1923.

HOULE, D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics*, **130**, 195–204.

JOLY, D. AND BRESSAC, C. 1994. Sperm length in Drosophilidae (Diptera): estimation by testis and receptacle lengths. *Int. J. Insect Morphol. Embryol*., **23**, 85–92.

LANDE, R. 1979. Quantitative genetic analysis of multivariate evolution, applied to brain: body size allometry. *Evolution*, **34**, 402–416.

LYNCH, M. AND WALSH, B. 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer, Sunderland, MA.

MACKAY, T. F. C. 1985. A quantitative genetic analysis of fitness and its componenets in *Drosophila melanogaster*. *Genet. Res*., **47**, 59–70.

MARKOW, T. A. AND HOCUTT, G. D. 1998. Reproductive isolation in Sonoran Desert *Drosophila*. In: Howard, D. J. and

Berlocher, S. H. (eds) *Endless Forms: Species and Speciation*, pp. 234–244. Oxford University Press, Oxford.

MATHER, K. AND JINKS, J. L. 1982. *Biometrical Genetics: the Study of Continuous Variation*. Chapman & Hall, New York.

MORROW, E. H. AND GAGE, M. J. G. 2000. The evolution of sperm length in moths. *Proc. R. Soc. B*, **267**, 307–313.

MOUSSEAU, T. A. AND ROFF, D. A. 1987. Natural selection and the heritability of fitness components. *Heredity*, **59**, 187–197.

PARTRIDGE, L. AND FARQUHAR, M. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. *Anim. Behav.*, **31**, 871–877.

PITNICK, S. 1996. Investment in testes and the cost of making long sperm in *Drosophila*. *Am. Nat.*, **148**, 57–80.

PITNICK, S., MARKOW, T. A. AND SPICER, G. S. 1995a. Delayed male maturity is a cost of producing large sperm in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.*, **92**, 10 614–10 618.

PITNICK, S., SPICER, G. S. AND MARKOW, T. A. 1995b. How long is a giant sperm? *Nature*, **375**, 109.

PITNICK, S., MARKOW, T. A. AND SPICER, G. S. 1999. Evolution of multiple kinds of female sperm-storage organs in *Drosophila*. *Evolution*, **53**, 1804–1822.

PRESGRAVES, D. C., BAKER, R. H. AND WILKINSON, G. S. 1999. Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. *Proc. R. Soc. B*, **266**, 1041–1047.

ROFF, D. A. 1992. *The Evolution of Life Histories: Theory and Analysis*. Chapman & Hall, New York.

SNOOK, R. R. 1997. Is the production of multiple sperm types adaptive? *Evolution*, **51**, 797–808.

STEARNS, S. C. 1992. *The Evolution of Life Histories*. Oxford University Press, Oxford.

TANTAWY, A. O. 1961. Effects of temperature on productivity and genetic variance of body size in populations of *Drosophila pseudoobscura*. *Genetics*, **46**, 227–238.

ZWAAN, B., BIJLSMA, R. AND HOEKSTRA, R. F. 1995. Artificial selection for development time in *Drosophila melanogaster* in relation to the evolution of aging: direct and correlated responses. *Evolution*, **49**, 635–648.