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Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies

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Sperm and female reproductive tract morphology are among the most rapidly evolving characters known in insects. To investigate whether interspecific variation in these traits results from divergent coevolution we examined testis size, sperm length and female reproductive tract morphology for evidence of correlated evolution using 13 species of diopsid stalk-eyed flies. We found that sperm dimorphism (the simultaneous production of two size classes of sperm by individual males) is ancestral and occurs in four genera while sperm monomorphism evolved once and persists in one genus. The length of 'long sperm' types, though unrelated to male body or testis size, exhibits correlated evolution with two regions of the female reproductive tract, the spermathecae and ventral receptacle, where sperm are typically stored and used for fertilization, respectively. Two lines of evidence indicate that 'short sperm', which are probably incapable of fertilization, coevolve with spermathecae. First, loss of sperm dimorphism coincides phylogenetically with reduction or loss of spermathecae. Second, evolutionary change in short-sperm length correlates with change in spermathecal size but not spermathecal duct length or ventral receptacle length. Morphological coevolution between sperm and female reproductive tracts is consistent with a history of female-mediated selection on sperm length.

Keywords: stalk-eyed flies; sperm; comparative method; sexual selection; sperm competition

1. INTRODUCTION

Sexual selection resulting from male competition or female choice has traditionally been invoked to explain the evolution of elaborate male secondary sexual traits such as large antlers or bright plumage (Darwin 1871; Andersson 1994). However, Parker (1970) first pointed out that male reproductive success can be influenced by post-copulatory events whenever sperm of two or more males compete for fertilization of eggs. As a consequence of such sperm competition, males are expected to maximize fertilization success by producing many, tiny gametes (Parker 1982, 1984). This prediction derives from two assumptions: (i) fertilization follows a 'raffle principle' in which a male's probability of fertilization is a function of his relative contribution to the total number of sperm in competition; and (ii) male reproductive resources are limited such that a trade-off occurs between the size and number of gametes produced (Parker 1982, 1984). The theory fares well in taxa with external fertilization (Stockley *et al.* 1997, 1998), but less so in those with internal fertilization. Instead, sperm morphology in internally fertilizing taxa, not unlike secondary sex traits, evolves rapidly and frequently takes on highly exaggerated sizes and forms (Franzen 1970; Sivinski 1980; Walker 1980; Keller & Reeve 1994; Pitnick *et al.* 1995). Similarly, male genitalia show greater morphological complexity in taxa with internal versus external fertilization (Eberhard 1985) and higher rates of divergence in species with polyandrous versus monandrous mating systems (Arnqvist 1998). Thus, in addition to sperm competition, the interaction of male genital and ejaculate characteristics (i.e. sperm and accessory substances) with the physical and biochemical environment of the female reproductive tract generates further opportunity for post-copulatory sexual selection by 'cryptic' female preferences (Sivinski 1980; Thornhill 1983; Eberhard 1985, 1996; Birkhead *et al.* 1993; Keller & Reeve 1994).

Female reproductive tracts show an exceedingly complex morphology, particularly in insects (Keller & Reeve 1994; Eberhard 1996). Growing evidence indicates that, after sperm transfer and prior to fertilization, female reproductive tracts can manipulate, expel, digest or nurture sperm in storage (see Eberhard 1996). Comparative studies demonstrating correlations between sperm length and female reproductive tracts in a wide variety of taxa (ptiliid beetles (Dybas & Dybas 1981), fleas (Rothschild 1991), *Drosophila* (Hihara & Kurukawa 1987; Pitnick & Markow 1994; Pitnick *et al.* 1999) and birds (Briskie *et al.* 1997)) are consistent with female-mediated selection on sperm morphology. In stalk-eyed flies (Diopsidae), sperm are usually transferred by spermatophores (Kotrba 1996) and stored in chitinous spermathecae or multichambered ventral receptacles (figure 1; Kotrba 1993, 1995). Detailed descriptive studies of female reproductive tracts in stalk-eyed flies have

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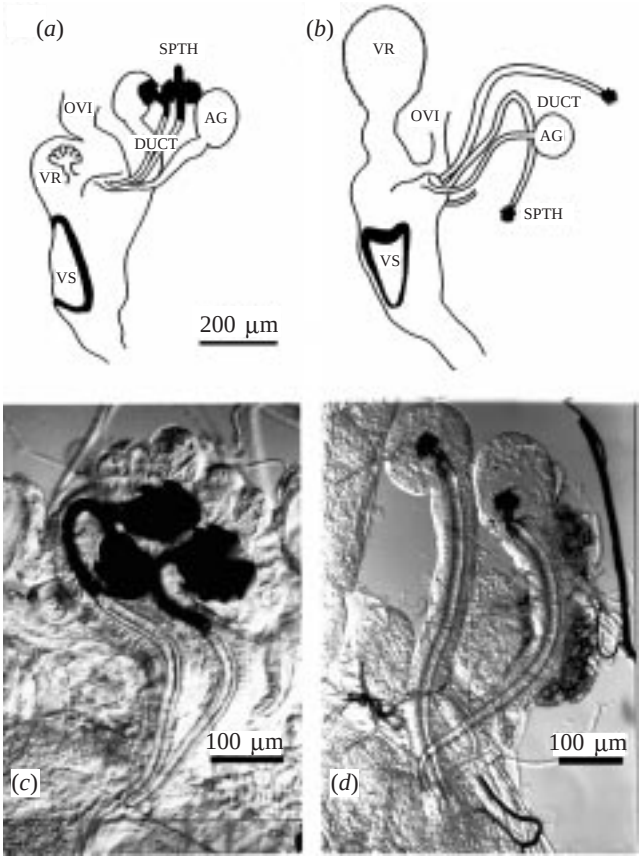

Figure 1. Schematic illustrations of the female reproductive tracts of (*a*) *C. whitei* and (*b*) *D. munroi*. AG, accessory gland; DUCT, spermathecal ducts; OVI, common oviduct; SPTH, spermathecae; VR, ventral receptacle; VS, ventral sclerite (after Kotrba 1993, 1995). Corresponding photo images of (*c*) the three spermathecae of *C. whitei*, and (*d*) the two relatively degenerate spermathecae of *D. munroi*.

revealed substantial variation in morphology indicating likely differences in patterns of sperm storage and usage among species (Kotrba 1993, 1995).

Here, we use the comparative method of analysing phylogenetically independent contrasts (Felsenstein 1985) to investigate whether sperm length in stalk-eyed flies exhibits correlated evolution with sexual dimorphism in eye span (Wilkinson & Dodson 1997), body size, testis size or size of three female reproductive tract regions. We found two apparently distinct sperm production strategies: (i) production of a single, very long sperm type, and (ii) sperm dimorphism—the simultaneous production of two types of sperm by individual males (see also Presgraves *et al.* 1997). Both strategies fail to conform to the 'many, tiny sperm' expectation and therefore require explanation. Exorbitant sperm lengths have evolved independently in ostracods (E. Foster, unpublished results, cited in Ladle & Foster 1992), beetles (Dybas & Dybas 1981; Taylor 1982), millipedes and waterbugs (Sivinski 1984) and multiple times within the genus *Drosophila* (Pitnick *et al*. 1995). Neither female- nor egg-provisioning functions readily explains the dramatic evolutionary increases in sperm length observed in *Drosophila* (Karr & Pitnick 1996; Pitnick *et al*. 1997). Production of multiple sperm types has also evolved numerous times in invertebrates (Eberhard 1996; Snook & Karr 1998). Evidence to date indicates that long-sperm morphs in sperm dimorphic species are fertilization competent while short apyrene sperm in Lepidoptera (Silberglied *et al.* 1984) and nucleated short sperm in sperm dimorphic *Drosophila* (Snook *et al.* 1994; Snook & Karr 1998) are not. Despite a long list of hypotheses suggesting adaptation to sperm competition (e.g. Silberglied *et al.* 1984; Sivinski 1984; Ladle & Foster 1992; see § 4), why males produce either one type of typically long sperm or two different lengths of sperm is not understood. In this paper we test whether these alternative sperm production strategies have evolved in response to change in male secondary sex traits which influence mating success or to anatomical change in regions of the female reproductive tract where sperm storage and fertilization occur.

2. MATERIAL AND METHODS

(a) *Data collection*

We randomly selected flies of each sex from laboratory populations of 12 species of stalk-eyed flies (family Diopsidae) for measurement. The outgroup representative, *Teleoglabrus milleri* (family Centrioncidae), was unavailable in the laboratory and we therefore measured a field-collected specimen of this species preserved in 95% ethanol. The phenotypic values and the number of individuals measured for each sex and species are presented in table 1. We used NIH Image v. 1.59 software to measure digitized video images captured from either dissecting or compound contrast microscopes. We anaesthetized flies with CO_2 and measured eye span and body length (from the head to the tip of the retracted wing) at $\times 40$ magnification. The degree of sexual dimorphism in eye span was calculated by subtracting the mean female eye span from the mean male eye span to provide a simple species-level index of the intensity of pre-copulatory sexual selection (Wilkinson & Dodson 1997). We added 1.0 to all dimorphism values prior to $\log_{10}$ transformation.

Testes were dissected from individual males of each species and immediately transferred to a droplet of phosphate-buffered saline solution (PBS, pH = 6.8) on a glass slide. The length of one randomly chosen testis was measured at $\times 100$. The other testis was gently ruptured to release sperm bundles (spermatocysts) and we measured the length of mature bundles. We visualized sperm bundles using phase contrast microscopy at $\times 200$. The mean sperm length for each male was calculated from the five longest, most mature sperm bundles present in the testis to control for the effects of development. For species with two classes of sperm size, we used the five longest sperm bundles for each class to calculate their respective means. When analysing sperm length across all species, we used the length of long sperm from sperm-dimorphic species and considered short-sperm types separately.

Sperm migrate from a spermatophore through spermathecal ducts to chitinized spermathecae and after storage are shunted to the ventral receptacle for fertilization (see figure 1 and Kotrba 1993, 1995, 1996). Under a coevolutionary scenario, sperm length might exhibit correlated evolutionary change with any one of these female anatomical regions. Reproductive tracts from females of each species were therefore dissected in Ringer's solution and transferred to a drop of 3:1 PBS:glycerol on a glass slide and measured at $\times 100$ magnification. The area of a single spermatheca and the lengths of the ventral receptacle and spermathecal ducts were measured for each female at $\times 100$ using phase contrast or Nomarski microscopy.

Table 1. *Species data*

(Phenotypic values are given as mean (s.e.; *n*).)

species	eye span (mm)		body length (mm)		testis length (mm)		long sperm length (mm)		short sperm length (mm)	
(*a*) male traits										
Cyrtodiopsis dalmanni	8.83	(0.13; 3)	7.17	(0.15; 3)	4.30	(0.59; 3)	0.176	(0.003; 3)	0.089	(0.003; 3)
Cyrtodiopsis whitei (Gombak)	9.56	(0.09; 100)	7.12	(0.05; 100)	3.06	(0.09; 3)	0.192	(0.001; 20)	0.057	(0.001; 20)
Cyrtodiopsis whitei (Chang Mai)	9.44	(0.09; 100)	7.42	(0.05; 100)	2.82	(0.08; 3)	0.190	(0.001; 20)	0.053	(0.002; 20)
Cyrtodiopsis quinqueguttata	4.37	(0.09; 3)	7.41	(0.24; 3)	3.96	(0.30; 3)	0.183	(0.001; 3)	0.087	(0.001; 3)
Teleopsis quadriguttata	3.99	(0.03; 3)	6.01	(0.02; 3)	2.21	(0.12; 3)	0.227	(0.006; 3)	0.096	(0.002; 3)
Diopsis apicalis	9.95	(0.11; 4)	8.32	(0.06; 4)	2.95	(0.20; 4)	0.419	(0.003; 2)	0.119	(0.008; 2)
Diopsis fumipennis	7.50	(0.06; 3)	7.44	(0.09; 3)	2.61	(0.16; 3)	0.465	(0.007; 1)	0.119	(0.009; 1)
Diasemopsis dubia	7.43	(0.09; 3)	6.76	(0.08; 3)	2.60	(0.04; 3)	1.454	(0.084; 3)	n/a	
Diasemopsis obstans	7.08	(0.13; 2)	6.70	(0.07; 2)	2.59	(0.33; 2)	1.618	(0.095; 2)	n/a	
Diasemopsis munroi sp. B	6.74	(0.30; 2)	6.26	(0.13; 2)	5.89	(0.42; 2)	0.920	(0.028; 2)	n/a	
Diasemopsis aethiopica	7.46	(0.08; 4)	7.38	(0.07: 4)	4.77	(0.07; 3)	2.988	(0.117; 3)	n/a	
Sphyracephala brevicornis	1.94	(0.07; 4)	5.35	(0.16; 4)	2.45	(0.17; 3)	0.497	(0.007; 2)	0.124	(0.011; 2)
Teleoglabrus milleri	1.06	(0.03; 2)	5.85	(0.10; 2)	1.27	(0.05; 2)	0.337	(0.001; 2)	0.112	(0.002; 2)

species	eye span (mm)		body length (mm)		spermatheca area (μm^2)		spermathecal duct length (mm)		ventral receptacle length (mm)	
(*b*) female traits										
Cyrtodiopsis dalmanni	5.98	(0.03; 3)	6.92	(0.09; 3)	1990	(52; 3)	0.296	(0.002; 3)	0.061	(0.003; 3)
Cyrtodiopsis whitei (Gombak)	6.03	(0.41; 3)	6.85	(0.32; 3)	1559	(213; 3)	0.284	(0.10; 3)	0.069	(0.002; 3)
Cyrtodiopsis whitei (Chang Mai)	5.74	(0.07; 3)	6.81	(0.10; 3)	1337	(8; 3)	0.275	(0.010; 3)	0.075	(0.001; 3)
Cyrtodiopsis quinqueguttata	4.56	(0.15; 3)	7.47	(0.12; 3)	2235	(64; 3)	0.356	(0.026; 3)	0.063	(0.001; 3)
Teleopsis quadriguttata	4.08	(0.03; 3)	6.19	(0.16; 3)	3640	(141; 3)	0.436	(0.006; 3)	0.080	(0.002; 3)
Diopsis apicalis	8.21	(0.15; 3)	8.28	(0.07; 3)	5243	(52; 3)	0.449	(0.021; 3)	0.115	(0.002; 3)
Diopsis fumipennis	6.32	(0.17; 3)	7.65	(0.14; 3)	5262	(148; 3)	0.379	(0.021; 3)	0.106	(0.002; 3)
Diasemopsis dubia	5.15	(0.09; 3)	6.58	(0.13; 3)	1834	(174; 3)	0.351	(0.015; 3)	0.231	(0.005; 3)
Diasemopsis obstans	4.63	(0.66; 2)	6.07	(0.56; 2)	1962	(480; 2)	—		0.213	(0.007; 2)
Diasemopsis munroi sp. B	4.77	(0.03; 3)	6.51	(0.02; 3)	207	(75; 3)	0.343	(0.021; 3)	0.210	(0.007; 3)
Diasemopsis aethiopica	5.96	(0.28; 3)	7.02	(0.22; 3)	5426	(296; 3)	0.812	(0.025; 3)	0.215	(0.010; 3)
Sphyracephala brevicornis	2.07	(0.03; 3)	5.84	(0.07; 3)	2660	(50; 3)	0.236	(0.004; 3)	0.105	(0.010; 3)
Sphyracephala becarri	2.16	(0.02; 3)	5.13	(0.03; 3)	2296	(125; 3)	0.127	(0.010; 3)	0.073	(0.002; 3)
Teleoglabrus milleri	—		—		—		—		—	

(b) *Statistical methods and comparative analysis*

The phylogenetic relationships between the species examined in this study (figure 2) were derived from a larger systematic analysis of 33 diopsid species and two outgroup taxa (Baker *et al*. 1999). Molecular sequence data were generated from three mitochondrial genes (cytochrome oxidase II, 12S ribosomal RNA and 16S ribosomal RNA) and three nuclear genes (elongation factor-1, *wingless* and *white*). These data comprise a total of 3236 characters of which 966 are phylogenetically informative. Details concerning sampling localities, DNA amplification, alignment and data analysis are provided in Baker *et al*. (1999). Parsimony analysis using PAUP* 4.0d64 (Swofford 1998) produced the single most parsimonious cladogram with a length of 3724 steps. Overall, branch support for relationships on this tree are strong. Twenty-one of the 32 nodes have bootstrap values (Felsenstein 1985) greater than or equal to 95 and Bremer (1994) support values greater than or equal to ten. The relationships specified in figure 2 are redrawn from this cladogram after removing inapplicable taxa.

We used Felsenstein's (1985) method of comparing phylogenetically independent contrasts to test for correlated evolution among characters. This method is superior to alternative methods (e.g. phylogenetic autoregression; Gittleman & Kot 1990) when relatively small phylogenies (around 15 taxa) are used (Purvis *et al*. 1994; Martins 1996). We calculated standardized independent contrasts from $\log_{10}$-transformed phenotypic values using the Comparative Analysis by Independent Contrasts (CAIC v. 2.0.0) program of Purvis & Rimbaut (1994) and tested for correlated evolution by fitting least-square linear regressions forced through the origin (Harvey & Pagel 1991; Garland *et al*. 1992). We assumed a gradual model of phenotypic evolution and therefore standardized independent contrasts with branch lengths (see Garland *et al*. 1992). A punctuated model of evolution gave qualitatitively similar results. For all analyses we tested the statistical assumptions of general linear models as well as the statistical and evolutionary assumptions of the independent contrasts method (see Purvis & Rimbaut 1994). In some cases branch length data were $\log_{10}$-transformed to meet the latter assumptions (see Garland *et al*. 1992; Purvis & Rimbaut 1994). Unless otherwise indicated, all statistical tests use independent contrasts.

3. RESULTS

(a) *Sperm length evolution*

Sperm length varied nearly 60-fold across the 13 species examined, with means ranging from 0.05 mm to

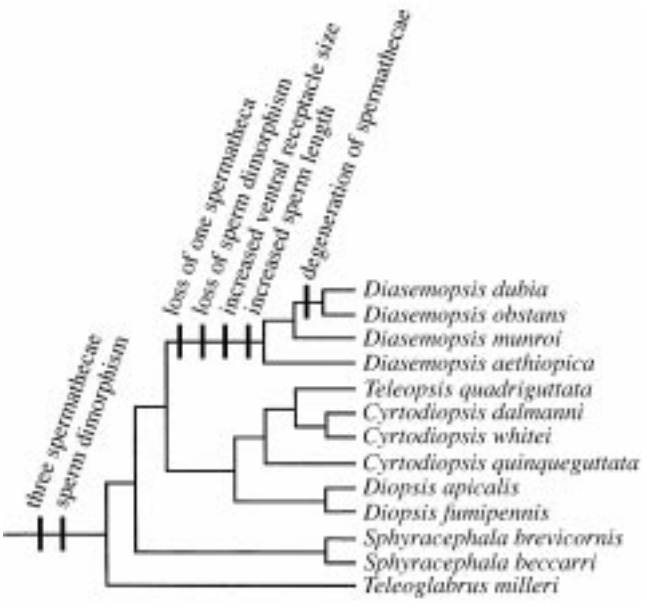

Figure 2. Maximum parsimony hypothesis of the phylogenetic relationships between 12 diopsid species and an outgroup species. The history of sperm and female reproductive tract character evolution, as inferred by parsimony, is indicated on the tree branches.

3 mm (table 1). Males of the African genus *Diasemopsis* produce a single type of sperm, the longest of which is nearly half the body length of *Diasemopsis aethiopica*. In contrast, males of the *Sphyracephala* and *Diopsis* (including *Diopsis*, *Teleopsis* and *Cyrtodiopsis*) clades simultaneously produce two classes of nucleated sperm that do not overlap in length (table 1). Assuming the most parsimonious history of character evolution, the presence of sperm dimorphism in the outgroup *T. milleri* indicates that sperm dimorphism was present in the common ancestor of diopsids but was lost in the ancestor to all *Diasemopsis* species (figure 2). Without exception, sperm lengths in sperm monomorphic species exceed those in sperm dimorphic species (Mann–Whitney $U_{10,4}=40.0$ and $p=0.005$; no phylogenetic correction).

No trait measured in males showed correlated evolution with sperm length. The degree of sexual dimorphism for eye span was unrelated to long- ($F_{1,12}=0.42$, $r^2=0.4$ and $p=0.53$) or short-sperm length ($F_{1,8}=1.26$, $r^2=0.15$ and $p=0.30$). Male body size and testis size were unrelated ($F_{1,13}=0.05$, $r^2=0.004$ and $p=0.83$) and neither male body size ($F_{1,13}=0.48$, $r^2=0.04$ and $p=0.48$) nor testis length ($F_{1,13}=0.21$, $r^2=0.02$ and $p=0.66$) were related to long-sperm length. Within sperm-dimorphic species, the length of short sperm did not show correlated evolution with body size ($F_{1,9}=0.89$, $r^2=0.10$ and $p=0.37$) or testis length ($F_{1,9}=1.09$, $r^2=0.12$ and $p=0.33$). Furthermore, the lengths of long and short sperm were not correlated ($F_{1,9}=0.80$, $r^2=0.09$ and $p=0.40$), indicating independent evolutionary change.

(b) *Sperm–reproductive tract coevolution*

The length of long sperm exhibited a significant pattern of correlated evolution with ventral receptacle length ($F_{1,12}=23.33$, $r^2=0.68$ and $p=0.0005$; $y=1.67x$) and spermathecal duct length ($F_{1,11}=6.38$, $r^2=0.39$ and $p=0.030$; $y=0.92x$) and nearly so with spermathecal area

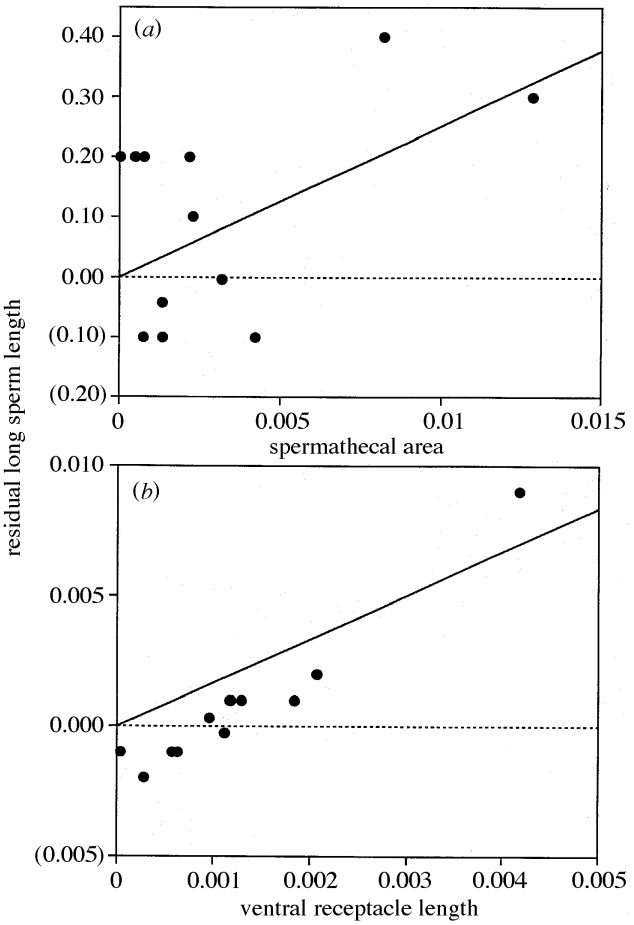

Figure 3. Relationships between (*a*) residual long-sperm length contrasts (from sperm length contrasts regressed on ventral receptacle length contrasts) and spermathecal area contrasts and (*b*) residual long-sperm length contrasts (from sperm length contrasts regressed on spermathecal area contrasts) and ventral receptacle length contrasts.

($F_{1,12}=3.78$, $r^2=0.26$ and $p=0.078$). The probabilities associated with the latter two analyses should be interpreted with caution because the assumptions of the independent contrasts method were violated, i.e. appropriate standardization of the contrasts was not possible using raw or transformed branch length data. To control for possible correlations among reproductive tract characters we used multiple regression with zero intercept (Harvey & Pagel 1991; Purvis & Rimbaut 1994) of sperm length on reproductive tract characters. This model explained 81% of the variation in long-sperm length (model, $F_{2,12}=21.87$ and $p=0.0002$) and included spermathecal area (figure 3*a*, $t=2.69$ and $p=0.023$) and ventral receptacle length (figure 3*b*, $t=5.48$ and $p=0.0003$) but not spermathecal duct length. This analysis satisfied all statistical and independent contrast assumptions.

Females of all *Diasemopsis* species have lost one spermatheca and the two remaining spermathecae of several *Diasemopsis* species appear to have lost sperm storage function (figures 1*b* and 2; Kotrba 1995). In contrast, females of all *Sphyracephala* and *Diopsis* species have retained the ancestral state of three functional spermathecae (as indicated by the outgroup *T. milleri*; see also Kotrba 1995). Sperm dimorphism, which occurs in *Diopsis* and *Sphyracephala* but not *Diasemopsis*, therefore

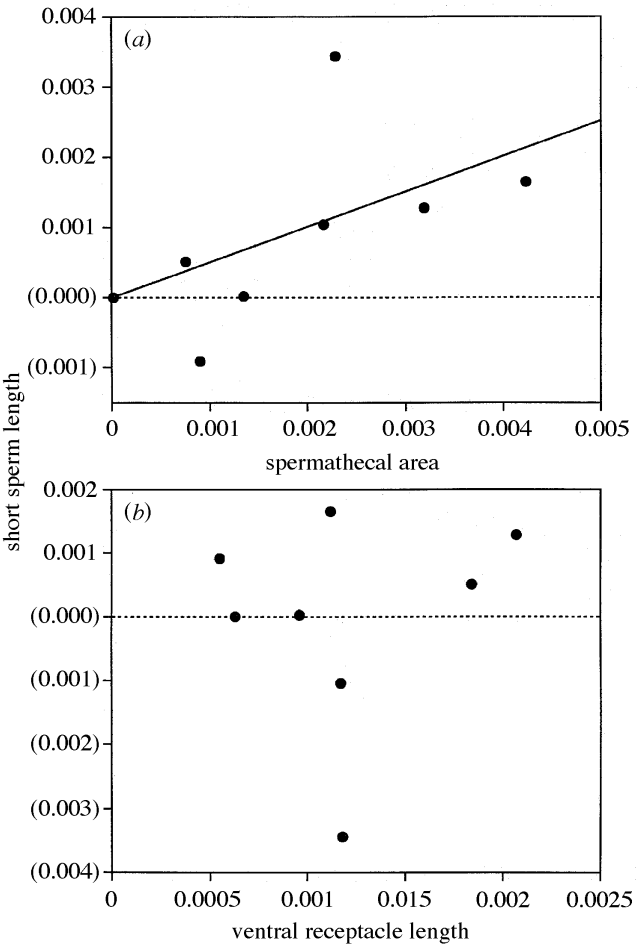

Figure 4. Relationships between short-sperm length contrasts and contrasts for (*a*) spermathecal area and (*b*) ventral receptacle length.

corresponds phylogenetically with the presence of functional spermathecae (figure 2). Unfortunately there were too few character transitions to analyse this relationship statistically. Nonetheless, a second pattern supported coevolutionary change: across sperm-dimorphic species, short-sperm length exhibited correlated evolution with spermathecal area (figure 4*a*, $F_{1,8}=9.31$, $r^2=0.57$ and $p=0.019$; $y=0.50x$). However, short-sperm length did not show correlated change with either spermathecal duct length ($F_{1,8}=0.33$, $r^2=0.05$ and $p=0.58$) or ventral receptacle length (figure 4*b*, $F_{1,8}=0.62$, $r^2=0.08$ and $p=0.46$).

Finally, no measured aspect of the reproductive tract was correlated with female body size: ventral receptacle length ($F_{1,12}=0.19$, $r^2=0.02$ and $p=0.67$); spermathecal duct length ($F_{1,11}=3.14$, $r^2=0.24$ and $p=0.11$); spermathecal area ($F_{1,12}=0.21$, $r^2=0.02$ and $p=0.66$).

4. DISCUSSION

Sperm lengths in stalk-eyed flies show clear evidence of coevolution with the functional morphology of female reproductive tracts. Indeed, the female reproductive tract is a better predictor of sperm length than any aspect of the male phenotype measured in this study. We found two discrete ejaculate strategies in stalk-eyed flies (long monomorphic sperm and sperm dimorphism) which are associated with different modes of sperm storage by females. The ancestral character states inferred from the outgroup *T. milleri*, sperm dimorphism and three functional spermathecae, persist in the *Sphyracephala* and *Diopsis* clades. In *Diasemopsis*, however, one spermatheca has been lost and in some species (e.g. *Diasemopsis munroi*, *Diasemopsis obstans* and *Diasemopsis silvatica*) the remaining two have degenerated (figure 2; see also Kotrba 1995). The reduction or loss of sperm storage function by spermathecae in the ancestor to *Diasemopsis* appears to coincide with the loss of sperm dimorphism and the evolution of very long sperm and hypertrophied ventral receptacles (figure 2).

A shift in the primary site of sperm storage by *Diasemopsis* females, from the spermathecae to the ventral receptacle, therefore appears to be related to the evolution of increased sperm length. Evolution of giant sperm lengths in the genus *Drosophila* is also correlated with seminal (ventral) receptacle length and these, rather than the spermathecae, are the primary sperm storage organs (Pitnick *et al.* 1999). Furthermore, our finding of smaller sperm sizes in sperm-dimorphic versus -monomorphic species is also paralleled in *Drosophila*. Comparison of published sperm lengths between sperm-monomorphic and -dimorphic *Drosophila* species (Markow 1996) shows that, with only two exceptions, sperm length in sperm monomorphic species is always greater than in dimorphic species (Mann–Whitney $U_{39,18}=695$ and $p<0.0001$; no phylogenetic correction). The remarkably convergent evolution of these alternative sperm production strategies in both stalk-eyed flies and *Drosophila* suggests that similar selective pressures may shape these phenotypes.

Despite recent efforts (Snook *et al.* 1994; Snook & Markow 1996; Snook & Karr 1998; Snook 1999; D. C. Presgraves, T. L. Karr and G. S. Wilkinson, unpublished results), the function of short non-fertilizing sperm in sperm-dimorphic species remains unknown. The occurrence of sperm dimorphism only in those stalk-eyed flies retaining three functional spermathecae, as well as evidence for correlated evolution between short sperm and spermathecal size but not any other character, strongly suggest that (i) short-sperm function localizes to these sperm storage organs, and (ii) the spermathecae are important agents of selection on short-sperm length. Given that short sperm are unlikely to be involved in fertilization (Presgraves *et al.* 1997), it is not surprising that they fail to evolve in concert with the ventral receptacle, i.e. that part of the female reproductive tract where sperm reside just prior to fertilization (Kotrba 1993). Experimental work in the sperm dimorphic stalk-eyed fly *Cyrtodiopsis whitei* suggests that the length of short sperm may be related to a male's ability to prevent fertilization or sperm transfer by later mating males, i.e.'sperm defensive' ability (D. C. Presgraves, T. L. Karr and G. S. Wilkinson, unpublished results). The results reported here are consistent with this finding but also with an alternative hypothetical function, namely that short sperm act as 'cheap fillers' of sperm storage organs designed to delay female remating (Silberglied *et al.* 1984). The cheap filler and sperm defence hypotheses are not mutually exclusive and the present findings do not allow us to distinguish between them because both functions may be mediated by the fit between short-sperm length and spermathecal

size. Whatever their function, the pattern of coevolution with female reproductive tracts provides strong evidence that short sperm interact with the female.

The adaptive significance of fertilizing sperm length has also been the subject of much speculation, most of which has given limited consideration to the role of female reproductive tracts. For example, longer sperm are believed to possess increased 'swimming speed', which might prove advantageous when racing against other sperm for access to unfertilized eggs (e.g. Gomendio & Roldan 1991). However, a simple relationship between swimming speed and sperm length was not found in a recent study in birds (T. R. Birkhead and F. Fletcher, unpublished results, cited in Briskie *et al.* 1997) and the positive evidence in mammals is based on limited data, i.e. five species and no phylogenetic correction (Gomendio & Roldan 1991). Moreover, the sperm sizes found in some stalk-eyed flies (see table 1) and many other arthropods easily exceed the total distance between the sites of sperm deposition and storage or the sites of storage and fertilization. Selection on swimming speed is therefore not a viable hypothesis for sperm length evolution in these taxa (Pitnick & Markow 1994).

Instead, the morphological coevolution observed between sperm and the female reproductive tract strongly suggests intersexual selection consistent with male–female conflict (Parker 1979; Holland & Rice 1998). Because sperm length evolution tracks change in the size of spermathecae and ventral receptacles, the female reproductive tract almost certainly mediates selection on sperm morphology by influencing opportunities for sperm competition. Evidence to date indicates that most if not all diopsid females mate multiple times (Wilkinson & Dodson 1997; Wilkinson *et al.* 1998). As we have shown, sperm dimorphism occurs in species with spermathecal sperm storage, while in contrast, sperm monomorphism occurs in species with reduced spermathecae and an enormous ventral receptacle. The ventral receptacles of *Diasemopsis* contain many rigid chambers each of which appears to house individual, helically coiled sperm (figure 1*b*; Kotrba 1995). Longer sperm and, in particular, tightly coiled sperm that maximize the contact area with the surface of chamber walls might be difficult to displace by later mating males (see also Dybas & Dybas 1981; Pitnick & Markow 1994). If sperm length (and coiling) is a male adaptation for exploiting extant reproductive tract structures at the expense of female fitness or control of paternity, then increases in the length or number of chambers in a ventral receptacle are likely female counter-adaptations. The combination of increasing ventral receptacle chamber length and number increases both the size and the number of sperm storage sites. Consequently, males would have to produce prodigious amounts of very long sperm to monopolize paternity (for similar arguments in birds see Briskie *et al.* (1997) and in *Drosophila* see Pitnick *et al.* (In press)).

Clearly, much experimental work on the interactions between sperm and reproductive tract morphology (e.g. Bishop 1996) and how these interactions influence fitness in both sexes is needed to decipher the physiological details of post-copulatory sexual selection. Nevertheless, the results reported here and in a growing number of other studies indicate that considerations of the evolutionary significance of sperm length cannot ignore the role of females.

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