EVOLUTION OF MULTIPLE KINDS OF FEMALE SPERM-STORAGE ORGANS IN *DROSOPHILA*

Scott Pitnick,[1] Therese Markow,[2,3] and Greg S. Spicer[4]

[1]*Department of Biology, Syracuse University, 108 College Place, Syracuse, New York 13244-1270*
E-mail: sspitnic@syr.edu
[2]*Department of Zoology, Arizona State University, Tempe, Arizona 85287-1501*
[4]*Department of Biology, San Francisco State University, San Francisco, California 94132-1722*

Abstract.—Females of all species belonging to the family Drosophilidae have two kinds of sperm-storage organs: paired spherical spermathecae and a single elongate tubular seminal receptacle. We examined 113 species belonging to the genus *Drosophila* and closely allied genera and describe variation in female sperm-storage organ use and morphology. The macroevolutionary pattern of organ dysfunction and morphological divergence suggests that ancestrally both kinds of organs stored sperm. Loss of use of the spermathecae has evolved at least 13 times; evolutionary regain of spermathecal function has rarely if ever occurred. Loss of use of the seminal receptacle has likely occurred only once; in this case, all descendant species possess unusually elaborate spermathecae. Data further indicate that the seminal receptacle is the primary sperm-storage organ in *Drosophila*. This organ exhibits a pattern of strong correlated evolution with the length of sperm. The evolution of multiple kinds of female sperm-storage organs and the rapidly divergent and correlated evolution of sperm and female reproductive tract morphology are discussed.

Key words.—Cryptic female choice, reproduction, sexual selection, sperm, sperm competition, spermatheca, vestigial.

Received December 15, 1998.   Accepted June 2, 1999.

Sperm must survive long enough within the reproductive tract of the female to ensure fertilization. For species in which insemination and fertilization are temporally unlinked, for example, where egg production is continuous or oviposition substrates are separate from mating sites or infrequently encountered, natural selection has favored mechanisms to extend the survival of sperm within females. In the majority of species with internal fertilization, with the exception of most mammals, females possess organs highly specialized for storing sperm (e.g., molluscs: Baur 1998; annelids: Adiyodi 1988; arachnids: Thomas and Zeh 1984; insects: Davey 1965; crustacea: Bauer and Martin 1991; poeciliid fish: Kadow 1954; amphibians: Boisseau and Joly 1975; reptiles: Olsson and Madsen 1998; birds: Shugart 1988; bats: Racey 1979). Insects frequently have a dorsal invagination of the vagina, called the bursa copulatrix, which receives the penis and stores sperm. Additionally, females of most insect species possess a variable number of spermathecae (one in most orders), which are strongly differentiated and typically highly sclerotized receptacles for sperm storage (Snodgrass 1935; Davey 1965; Wigglesworth 1965). The causes of interspecific divergence in sperm-storage organ size, number, or shape are not well understood.

Variation in size or number may reflect differential storage capacity demands arising through divergence in female longevity, egg productivity, or sperm utilization efficiency or through differences in the costs of remating relative to the costs of maintaining sperm viability. In addition, sperm size and shape can differ substantially among species (e.g., Pitnick et al. 1995a), and selection for functional design may dictate that sperm-storage organ size matches sperm morphology in some respect.

It is also likely that sexual selection has featured promi-

nently in the divergence of female sperm-storage organ morphology. Because females typically remate before exhausting the supply of viable stored sperm, the sperm of successive males can compete for fertilizations (Parker 1970) or be differentially used by the female (Eberhard 1996; Birkhead 1998). Although a diverse array of male- and/or female-mediated processes may influence patterns of paternity, the morphology of the female reproductive tract will almost certainly be an important determinant of the sperm precedence pattern in many species. Consequently, differential selection on females to control paternity and alternative responses to such selection may have contributed to interspecific variation in sperm-storage organ morphology (Walker 1980; Briskie and Montgomerie 1993; Keller and Reeve 1995; Eberhard 1996; Otronen et al. 1997; Ward 1998). Unfortunately, with few exceptions (Siva-Jothy 1987; Gack and Peschke 1994) the functional relationship between female reproductive morphology and sperm precedence pattern is unknown.

One response to postcopulatory sexual selection on females to control paternity may be to increase the number of sperm-storage organs (Hellriegel and Ward 1998). By spatially separating the sperm of different mates, females can postpone until the moment of fertilization their choice among potential sires for their offspring. This would enhance a female's fitness if there is adaptive matching between variable male genotypes in the population and variable environmental conditions affecting larval development and if these conditions are not known to the female prior to oviposition (Ward 1998). Recent evidence supports this explanation for the maintenance of multiple spermathecae in dungflies. Female dungflies have three spermathecae, which appear to be organized into two functional units: a singlet and a doublet; the doublet being two spermathecae with a common epithelial envelope (Ward 1993). Females can differentially store the sperm of different males within these organs (Otronen et al. 1997) and appear to do so using male phenotypic cues cor-

[3] Present address: Center for Insect Science and Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721; E-mail: tmarkow@arl.arizona.edu.
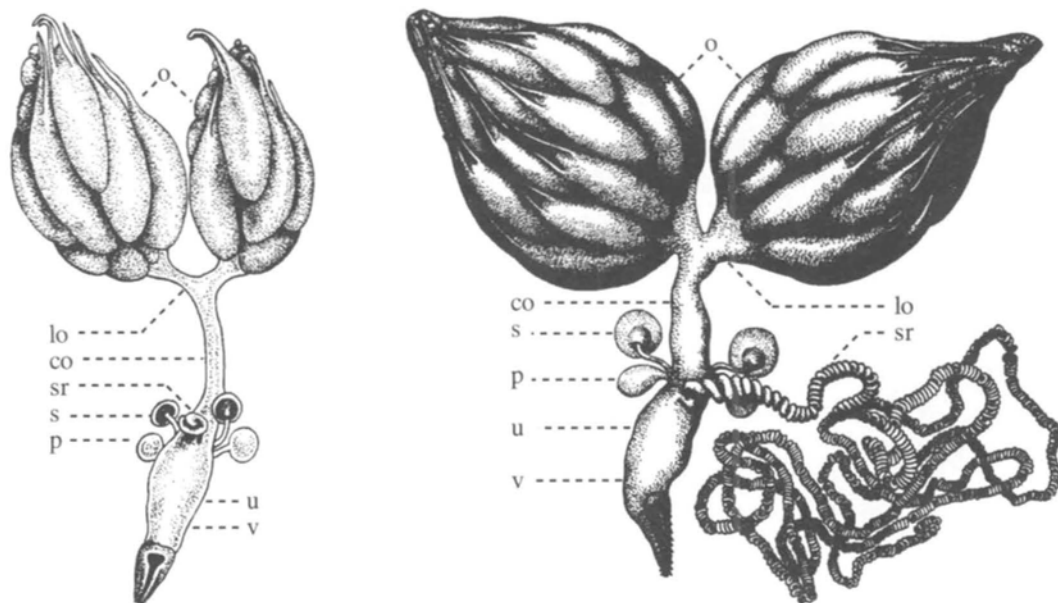
FIG. 1. Female reproductive tracts of *Drosophila pseudoobscura* (left) and *D. bifurca* (right), drawn to the same scale. *Drosophila psuedoobscura* has a 0.41-mm-long seminal receptacle and *D. bifurca* has an 81.67-mm-long seminal receptacle. Abbreviations: o, ovaries; lo, lateral oviduct; co, common oviduct; u, uterus; v, vagina; s, spermatheca; sr, seminal receptacle; p, parovarium. (Adapted with permission from Patterson 1943.)

related with single-locus variation for the enzyme phosophoglucomutase (*Pgm*). When fertilizing eggs, females tend to use sperm with the *Pgm* allele that optimizes larval development. Which allele is optimal differs depending upon whether the dungpat is in direct sunlight or shade (Ward 1998), however, this adaptive explanation for multiple sperm storage sites does not, however, explain why female dungflies have two morphologically distinct storage sites.

Postcopulatory sexual selection may further drive the evolution of female reproductive morphology to the extent that the female tract interacts with male traits directly involved in the acts of copulation, insemination, and fertilization. Evolutionary change in female morphology may arise to enhance matching or acceptance of male traits associated with superiority in gaining fertilization. Resulting directional changes in female morphology, and hence postcopulatory female choice, may result in runaway processes involving male genitalic, ejaculatory, or sperm traits (Eberhard 1985, 1996; Keller and Reeve 1995). Alternatively, coevolution of female reproductive morphology and male traits may arise through sexual conflict, with conflict between the sexes over sperm use inherent in the competition among males to fertilize the ova of multiply mating females (Knowlton and Greenwell 1984; Parker 1984; Davies 1989; Smuts and Smuts 1993; Rowe et al. 1994; Stockley 1996; Alexander et al. 1997; Brown et al. 1997; Gowaty 1997; Rice 1997; Gowaty and Buschhaus 1998). For example, conflict over the removal or repositioning of stored sperm within a female could select for male genitalia better designed to penetrate the female's sperm-storage organs as well as female organs that are more resistant to male intrusion. Such antagonistic coevolution may be ubiquitous, influencing female reproductive morphology and physiology and male traits such as genitalia (Eberhard 1985, 1996), ejaculatory secretions (Chapman et

al. 1995; Rice 1996), and sperm length (Briskie and Montgomery 1993).

The present study investigates the evolution of multiple kinds of female sperm-storage organs within the genus *Drosophila* and some closely allied genera. Because of the availability of numerous species for examination and the vast literature on their reproductive morphology and phylogenetics, *Drosophila* species, all of which possess two distinct sperm-storage organ types, provide a valuable opportunity for this kind of comparative exploration. Females of all species possess a pair of spermathecae and single seminal receptacle (also referred to as a ventral receptacle) (Fig. 1). The spermathecal ducts arise separately from a small, chitinized plate in the anterodorsal uterine wall. Each duct ends in a typically highly chitinized capsule, where the sperm are stored, surrounded by a cellular envelope (Sturtevant 1925, 1926). The seminal receptacle is a typically slender, blindended tubule arising from the anteroventral portion of the uterus (Nonidez 1920). Here we examine four nonmutually exclusive hypotheses to explain the evolution of multiple female sperm-storage organs in *Drosophila*. (1) Multiple organ types may have evolved to prevent males from directly accessing previously stored sperm. Alternatively, multiple organ types may be the result of selection for organ specialization to more than one function. Specifically, (2) one organ type may serve as a quarantine chamber for recent ejaculates or (3) one organ type may specialize in short-term sperm storage and the other in long-term storage. (4) Finally, multiple sperm-storage organs may be a consequence of the recent evolutionary innovation of a improved sperm-storage organ that coexists with a more ancestral organ type.

Among *Drosophila* species, sperm length has also been rapidly divergent (e.g., Pitnick et al. 1995a). Here we determine the extent of correlated evolution between the length

of sperm and length of the seminal receptacle. After providing evidence that the seminal receptacle is the primary female sperm-storage organ for the majority of *Drosophila* species, we address four alternative hypotheses to explain the pattern of correlated evolution between these male and female traits: (1) sequential evolution of female morphology due to utilitarian demands of sperm storage; (2) sexual conflict over sperm use; (3) good genes; and (4) runaway selection models of postcopulatory sexual selection.

MATERIALS AND METHODS

Species and Culturing

Species collection information is provided for *D. melanogaster, D. wassermani, D. nannoptera, D. pachea,* and *D. acanthoptera* by Pitnick et al. (1991); for *D. nigrospiracula* by Polak (1993); and for *D. subpalustris, D. recens, D. guttifera,* and *D. putrida* by Spicer and Jaenike (1996); *D. arizonae* was collected by T. A. Markow in San Carlos, Sonora, Mexico in May 1988. All remaining species examined were obtained from the National Drosophila Species Resource Center (Bowling Green State University, Bowling Green, OH); stock numbers are available from S. Pitnick upon request.

All flies were reared under uncrowded conditions on medium in either 200-ml bottles or 8-dram shell vials with live yeast at 24 ± 1°C at an approximate 12L:12D photoperiodic cycle and 1:1 sex ratio. The culture medium used, standard banana medium, cornmeal-agar-molasses medium, or instant *Drosophila* medium (Formula 4-24, Carolina Biological Supply Co., Burlington, NC), varied among species and may have been additionally modified depending upon their unique culturing needs (e.g., see Pitnick et al. 1997).

Measurement of Traits

Gross morphology of female sperm-storage organs and the location of sperm storage within females were determined by dissection of reproductively mature females taken from population bottles. Intact reproductive tracts were dissected into phosphate-buffered saline (PBS) on a glass slide. The paired spermathecae and the seminal receptacle of each female (see Fig. 1) were examined at 200× using phase contrast microscopy. Compression by the cover glass ruptured the spermathecae and compressed the seminal receptacles, thus permitting easy determination as to whether the organs contained sperm. Numerous females (> 10), each containing a copious supply of stored sperm, were examined for each species.

Determination of use and disuse of each organ type was based on the presence/absence of sperm and organ morphology. Such determination was unambiguous for spermathecae due to the absolute correlation between the absence of sperm and the degenerative or vestigial morphology of these organs. For all species categorized as having nonfunctional spermathecae, no females were observed to have sperm in these organs. Additionally, in all of these species the spermathecae were degenerative in appearance (for a more detailed description, see Results: Variation in Spermathecae). Although spermathecal size was not quantified, the difference in capsule size between species that use the spermathecae

and closely related species which do not was discrete and obvious. By contrast, for all species in which the spermathecae appeared large enough to be functional, some females were always found that contained sperm in these organs.

This protocol was employed in the examination of 102 species (Fig. 2). Eleven species additionally included in this study are, to our knowledge, not currently being cultured and thus were not directly examined. They were included because of the availability of detailed morphological description. The vestigial structure of the spermathecae of *D. ritae* and their disuse in sperm storage was described by Patterson (1947). Our interpretation of the pattern of sperm storage in *D. neohydei* and *D. paracanalinea* is based on drawings and description by Throckmorton (1962); interpretation for eight of the nine *fasciola* group species (*D. fulvalineata, D. pictilis, D. mojuiodes, D. moju, D. paraguttata, D. coroica, D. fasciola,* and *D. pictura* is based on drawings by Wasserman (1962), drawings and description by Throckmorton (1962), and by direct examination of the ninth species, *D. ellisoni.*

To examine the pattern of correlated evolution between the length of the seminal receptacle and the length of sperm, seminal receptacles were measured for 46 of the species (Fig. 3) considered in this study (*n* = 10 females per species). Seminal receptacles of most species are highly coiled and therefore must be uncoiled prior to measurement. The coils are maintained through binding by trachea and tracheoles; these were either removed by forceps or broken by gently tugging on two distal points of the seminal receptacle using two forceps. After uncoiling the seminal receptacle, the entire organ, still attached to the uterus, was transferred from PBS to white paraffin oil, which facilitated straightening the organ out so that it could be measured using the ocular micrometer of a dissecting microscope. The majority of sperm and female thorax length measures reported in Figure 3 were reported previously in Pitnick et al. (1995a). Novel sperm and thorax length data presented here for *D. nigrohydei, D. leonis, D. camargoi, D. canalinea,* and *D. bromeliae* were obtained using methods described by Pitnick and Markow (1994a).

Phylogenetic and Statistical Analyses

The phylogeny was compiled from a number of sources. The higher-level relationships were inferred from several published morphological (Grimaldi et al. 1992; Throckmorton 1975) and molecular (Beverley and Wilson 1982, 1984; Spicer 1988; Sullivan et al. 1990; Caccone et al. 1992; DeSalle 1992; Pelandakis and Solignac 1993; Kwiatowski et al. 1994; Powell and DeSalle 1995; Russo et al. 1995; Powell 1997) datasets, some of which were reanalyzed to construct the figures. In addition to the published sources, we used an unpublished dataset consisting of 2.7 Kb of nuclear large-subunit (28S) ribosomal RNA sequence (C. Bell, C. Saux, and G. S. Spicer, unpubl. data). The lower-level relationships were determined both by published sources and by our unpublished DNA sequence comprising about 1.5 Kb of the mitochondrial cytochrome oxidase subunits (G. S. Spicer, unpubl. data). Phylogenetic relationships for *D. melanogaster* (Ashburner 1989), *D. obscura* (Barrio et al. 1994), and *D. quinaria* (Spicer and Jaenike 1996) species groups were inferred entirely from the literature. The *D. virilis* (Spicer 1991,
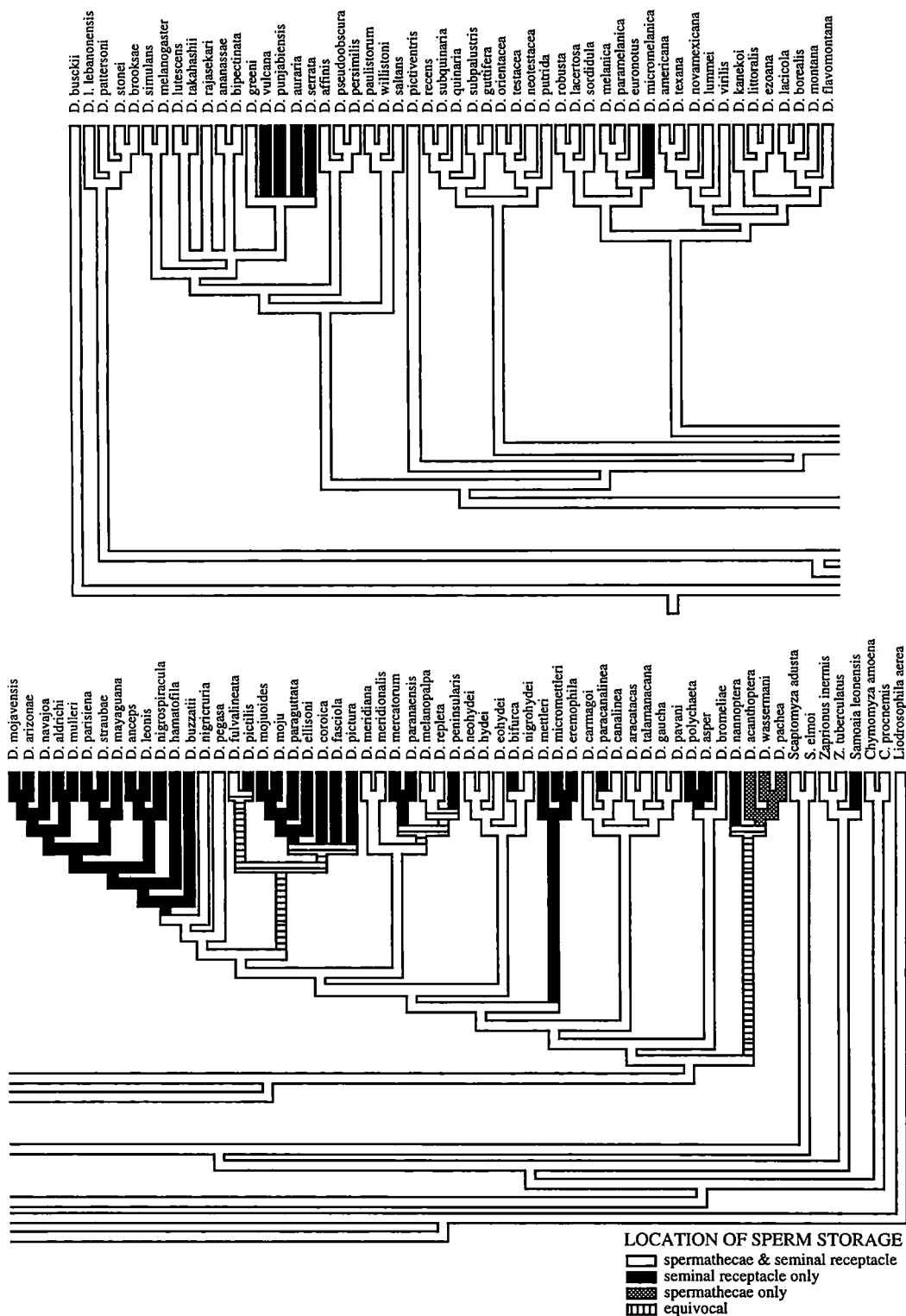
FIG. 2. The phylogeny and location of female sperm storage for 113 species of *Drosophila*.

1992) and *D. repleta* (Wasserman 1992; Spicer and Pitnick 1996) species groups were determined by using a combination of published phylogenies and by our sequencing studies. Relationships for the *D. nannoptera* and *D. melanica*

species groups were inferred entirely from our sequencing studies. Details of the phylogeny will be published elsewhere.

The tree and character mapping manipulations were accomplished using the program MacClade (Maddison and

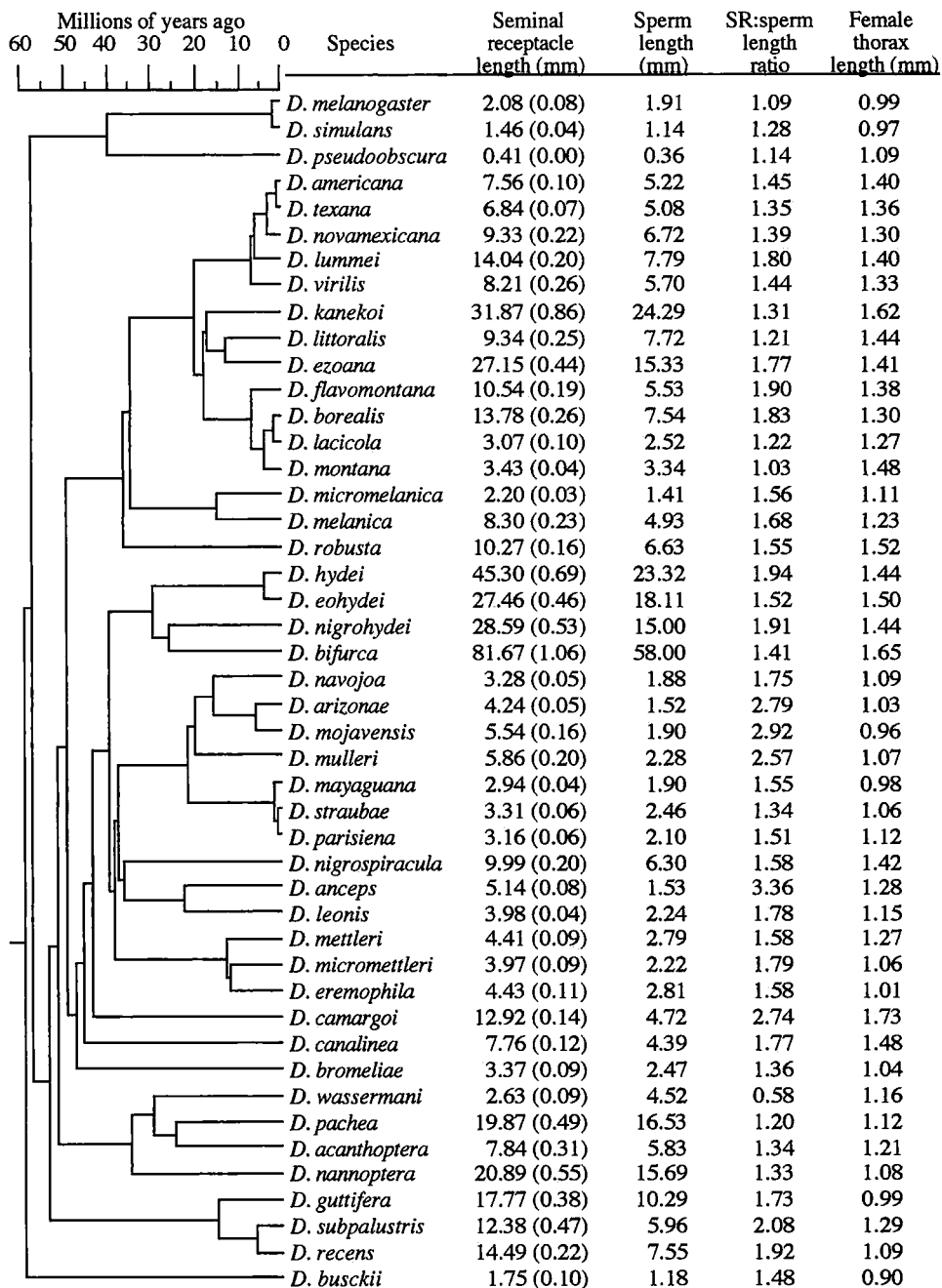| Species	Seminal receptacle length (mm)	Sperm length (mm)	SR:sperm length ratio	Female thorax length (mm)
D. melanogaster	2.08 (0.08)	1.91	1.09	0.99
D. simulans	1.46 (0.04)	1.14	1.28	0.97
D. pseudoobscura	0.41 (0.00)	0.36	1.14	1.09
D. americana	7.56 (0.10)	5.22	1.45	1.40
D. texana	6.84 (0.07)	5.08	1.35	1.36
D. novamexicana	9.33 (0.22)	6.72	1.39	1.30
D. lummei	14.04 (0.20)	7.79	1.80	1.40
D. virilis	8.21 (0.26)	5.70	1.44	1.33
D. kanekoi	31.87 (0.86)	24.29	1.31	1.62
D. littoralis	9.34 (0.25)	7.72	1.21	1.44
D. ezoana	27.15 (0.44)	15.33	1.77	1.41
D. flavomontana	10.54 (0.19)	5.53	1.90	1.38
D. borealis	13.78 (0.26)	7.54	1.83	1.30
D. lacicola	3.07 (0.10)	2.52	1.22	1.27
D. montana	3.43 (0.04)	3.34	1.03	1.48
D. micromelanica	2.20 (0.03)	1.41	1.56	1.11
D. melanica	8.30 (0.23)	4.93	1.68	1.23
D. robusta	10.27 (0.16)	6.63	1.55	1.52
D. hydei	45.30 (0.69)	23.32	1.94	1.44
D. eohydei	27.46 (0.46)	18.11	1.52	1.50
D. nigrohydei	28.59 (0.53)	15.00	1.91	1.44
D. bifurca	81.67 (1.06)	58.00	1.41	1.65
D. navojoa	3.28 (0.05)	1.88	1.75	1.09
D. arizonae	4.24 (0.05)	1.52	2.79	1.03
D. mojavensis	5.54 (0.16)	1.90	2.92	0.96
D. mulleri	5.86 (0.20)	2.28	2.57	1.07
D. mayaguana	2.94 (0.04)	1.90	1.55	0.98
D. straubae	3.31 (0.06)	2.46	1.34	1.06
D. parisiena	3.16 (0.06)	2.10	1.51	1.12
D. nigrospiracula	9.99 (0.20)	6.30	1.58	1.42
D. anceps	5.14 (0.08)	1.53	3.36	1.28
D. leonis	3.98 (0.04)	2.24	1.78	1.15
D. mettleri	4.41 (0.09)	2.79	1.58	1.27
D. micromettleri	3.97 (0.09)	2.22	1.79	1.06
D. eremophila	4.43 (0.11)	2.81	1.58	1.01
D. camargoi	12.92 (0.14)	4.72	2.74	1.73
D. canalinea	7.76 (0.12)	4.39	1.77	1.48
D. bromeliae	3.37 (0.09)	2.47	1.36	1.04
D. wassermani	2.63 (0.09)	4.52	0.58	1.16
D. pachea	19.87 (0.49)	16.53	1.20	1.12
D. acanthoptera	7.84 (0.31)	5.83	1.34	1.21
D. nannoptera	20.89 (0.55)	15.69	1.33	1.08
D. guttifera	17.77 (0.38)	10.29	1.73	0.99
D. subpalustris	12.38 (0.47)	5.96	2.08	1.29
D. recens	14.49 (0.22)	7.55	1.92	1.09
D. busckii	1.75 (0.10)	1.18	1.48	0.90

FIG. 3. The topology, estimated divergence times, and means for seminal receptacle length, sperm length, ratio of seminal receptacle to sperm length, and female thorax length for 46 species of *Drosophila*. Numbers in parentheses are standard errors. Standard errors for sperm and female thorax length data are reported in Pitnick et al. 1995a. See text for details. The scale bar represents time since divergence.

Maddison 1997). Before comparatively examine evolutionary relationships between characters, it was necessary to control for phylogenetic effects (Felsenstein 1985; Harvey and Pagel 1991). We therefore used Felsenstein's (1985) method of phylogenetically independent contrasts, which provides statistical independence of datapoints. Independent contrasts were computed (using the phylogenetic topology and branch lengths presented in Fig. 3) using the phenotypic diversity analysis program of Garland et al. (1993) and the CMSINGLE progam of Martins and Garland (1991). Standardization was accomplished by dividing each contrast by its standard deviation (the square root of the sum of its branch lengths) (Garland et al. 1992). Adequacy of this procedure was verified by a lack of significant linear or nonlinear trends in plots of the absolute value of each standardized independent contrast versus its standard deviation (Garland et al. 1992, 1993). The analyses presented employ a model that assumes gradual evolutionary change in variables, with branch lengths equal to estimated times of divergence (Felsenstein 1985; Martins and Garland 1991). Conclusions did not change qualitatively

when a punctuational model of evolutionary change was assumed (i.e., all branch lengths equal) (Martins and Garland 1991) or when "minimum evolution" methods were used (Martins and Garland 1991).

Because none of the variables examined in this study could be measured entirely without error, all reported slopes describing relationships among characters were derived by reduced major axis (RMA) regressions through the origin, using standardized independent contrasts of characters. RMA slopes were calculated as b/r, where b = slope from linear regression analysis and r = correlation coefficient (Garland 1985; Garland et al. 1992). The allometric nature of the relationships between seminal receptacle length and sperm length, as determined by the RMA slope, was compared with a null hypothesis slope of 1.0 using the test statistic provided by Clarke (1980) with degrees of freedom computed from his equation (5.1).

RESULTS

Location of Sperm Storage

Females of all *Drosophila* species examined possessed a pair of spermathecae and a single seminal receptacle (Fig. 1), although both organ types were not used for sperm storage in all species. The *Drosophila* phylogeny (Fig. 2) suggests that use of both organ types for sperm storage represents the ancestral condition in the genus. However, the evolution of these organs can be viewed quite differently depending upon the assumptions used to model character transition.

Because it is possible for character-state distributions on a most parsimonious tree to have multiple reconstructions (Swofford and Maddison 1987), there are a variety of ways to reconstruct the loss or regain of the use of the spermatheca as a sperm-storage organ among the *Drosophila* species examined (Fig. 2). The two most commonly employed algorithms used for determining reconstructions are Deltran (delayed change) and Acctran (accelerated change), each of which generates a slightly different evolutionary interpretation for these results. With the Deltran reconstruction the hypothesized change would infer that the use of the spermatheca as a sperm-storage organ has been lost 13 times (this is the same reconstruction as for Dollo parsimony). The Acctran reconstruction would suggest that the use of the spermatheca as a sperm-storage organ has been lost 11 times, but then regained as a sperm-storage organ in two instances.

These reconstructions are based on the assumption that the character-state changes are unordered and equally weighted, which may be the simplest model of character-state change, but not necessarily the most appropriate (Cunningham et al. 1998). Therefore, we performed a sensitivity analysis on the loss and gain of spermathecal use by using step-matrices to test the directionality of these changes (Donoghue and Ackerly 1996; Omland 1997; Ree and Donoghue 1998). Given that the change of spermathecal use appears to be toward loss, we investigated the ability to regain spermathecal use. By weighting a gain twice as likely as a loss, we found that the loss of spermathecal use is hypothesized to have occurred four times, but now the regain of the spermatheca as a sperm-storage organ has arisen between 11–12 times, depending on the reconstruction. If the weight is increased to have a gain

TABLE 1. Mean and standard error (in parentheses) dimensions of the seminal receptacle lumen for members of the nannoptera species group. Five equidistant measures were made on uncompressed organs for three females of each species.

Species	Smallest diameter (μm)	Largest diameter (μm)
Drosophila nannoptera	4.94 (.32)	12.99 (.54)
D. wassermani	5.06 (.22)	14.22 (.94)
D. pachea	3.74 (.25)	4.94 (.61)
D. acanthoptera	1.20 (.00)	2.29 (.24)

three times as likely as a loss, then the hypothesized loss of spermathecal use would have occurred only three times, with the regain of spermathecal use occurring 13–15 times. It is necessary to weight a gain to five times that of a loss for nonuse of the spermatheca as a sperm-storage organ to be interpreted as the ancestral condition. We have no way of evaluating the validity of these alternative weighting schemes other than to suggest that the regaining of a complex organ system like the spermatheca multiple times seems biologically less likely than their recurring loss (Maddison 1994). Until further information becomes available, the unordered, equally weighted parsimony approach is presumed to most conservatively represent the evolution of spermathecal use among *Drosophila* species.

In contrast to spermathecae evolution, loss of use of the seminal receptacle for sperm storage appears to be a rare event observed only in three species: *D. wassermani*, *D. pachea*, and *D. acanthoptera*. Phylogenetically these three species are members of a single lineage, the *D. nannoptera* species group, suggesting that such a loss occurred only once (Fig. 2). Contrary to previous reports (Jefferson 1977; Pitnick and Markow 1994a; Russell et al. 1977), disuse of the seminal receptacle in *D. wassermani* and *D. pachea* is not absolute. Of 30 females examined from mass matings for each species, 20.0% (6/30) of *D. pachea* females had sperm in the receptacle (seminal receptacle of five females contained three to five sperm each; seminal receptacle of one female contained one sperm) and 16.7% (5/30) of *D. wassermani* females had sperm in the seminal receptacle (seminal receptacle of three females contained two to five sperm each; seminal receptacles of two females contained eight to 15 sperm). No *D. acanthoptera* females (0/30) had sperm in their seminal receptacles, whereas the spermathecae in all of these females contained many sperm. No *D. nannoptera* females (0/30) contained sperm in the spermathecae, whereas all had sperm in the seminal receptacle.

Measurement of the dimensions of the seminal receptacles of *D. pachea*, *D. acanthoptera*, and *D. wassermani* provide proximate explanations for why this organ is predominantly dysfunctional in these species. Relative to the seminal receptacles of *D. nannoptera*, those of the other three species were either too short (*D. wassermani*; Fig. 3) or possessed too narrow of a lumen (*D. pachea* and *D. acanthoptera*; Table 1) to effectively store sperm.

Another unusual character-state transition observed is the use of the paired parovaria (see Fig. 1) for sperm storage in *D. nigricruria*. The parovaria of this species are unusually large, at least twice as large as those observed in any other
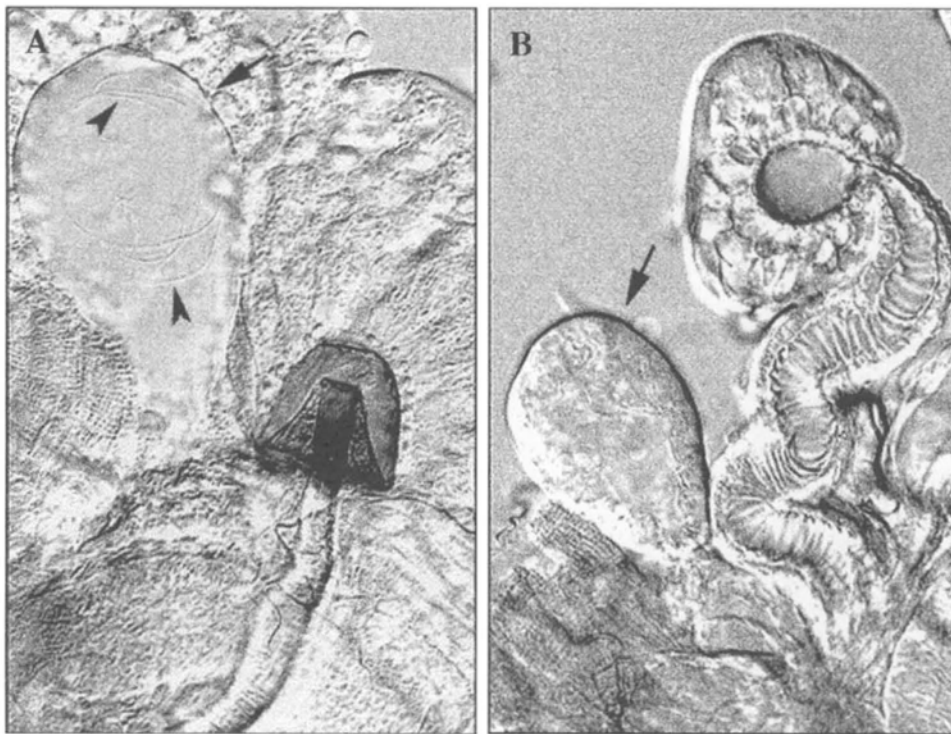
FIG. 4. Photomicrographs of a spermatheca and parovarium (arrows) of (A) *Drosophila nigricruria* and (B) *D. nigrospiracula*. Arrowheads indicate sperm stored within the lumen of the parovarium.

of the species examined. Figure 4 illustrates the large dimension of these organs in comparison with those of a related species, *D. nigrospiracula*, photographed at the same magnification. Of eight *D. nigricruria* females examined, five had motile sperm in both parovaria and three had motile sperm in one of the two parovaria, in addition to the seminal receptacle and spermathecae (Fig. 4). The lumen of the parovaria in this species are an estimated 10× that of the spermathecae.

Variation in Seminal Receptacles and Correlated Trait Evolution

Seminal receptacles varied across examined species in length by nearly 200 times (range 0.41–81.67 mm; Figs. 1, 3). The longest seminal receptacle, found in *D. bifurca*, was approximately 20 times longer than the total body length of the females bearing them. Although no attempt at quantification was made, a great variety of coiling and folding patterns was also observed among the seminal receptacles of different species. Phylogenetic trends in these patterns were evident, as illustrated by Throckmorton (1962, fig. 40).

Analysis of phylogenetically independent contrasts revealed that seminal receptacle length and sperm length in the genus *Drosophila* have evolved in a positively correlated fashion ($F = 396.22$; df $= 1, 44$; $r^2 = 0.900$; $P < 0.0001$). Because contrasts in female thorax length exhibited a significant positive relationship with contrasts in sperm length ($F = 4.74$; df $= 1, 44$; $r^2 = 0.097$; $P = 0.035$) and a marginally nonsignificant relationship with contrasts in seminal receptacle length ($F = 3.54$; df $= 1, 44$; $r^2 = 0.074$; $P = 0.066$), we performed a residual analysis on the relationship

between seminal receptacle length and sperm length to control for female body size effects in addition to phylogenetic effects (Fig. 5; $F = 365.84$; df $= 1, 44$; $r^2 = 0.893$; $P < 0.0001$). We believe the significant relationship between contrasts in seminal receptacle length and contrasts in thorax length to be due principally to colinearity with sperm length because most of the variation in seminal receptacle length explained by body size disappeared after controlling for sperm length by residual analysis ($F = 0.257$; df $= 1, 44$; $r^2 = 0.006$, $P = 0.614$). The significant correlation between contrasts in sperm length and contrasts in female size, in turn, is likely the result of genetic correlation between male and female size, as there is a highly significant relationship between sperm length and male body size evolution in *Drosophila* (Pitnick 1996; Pitnick et al. 1995a).

With the exception of *D. wassermani*, which rarely uses the seminal receptacle for sperm storage, seminal receptacle length exceeded sperm length in the remaining 45 species examined. The ratio of seminal receptacle to sperm length varied from 1.03 to 3.36 with a mean (± SE) of 1.66 ± 0.08. The significantly positive allometric scaling of contrasts in residual seminal receptacle length on contrasts in residual sperm length (Fig. 5A; RMA slope $= 1.64$; $t = 3.57$, df $= 32$, $P < 0.01$) indicates that relatively large changes in sperm length (in this case, increases) are associated with even greater relative increases in seminal receptacle length. Thus, the present analysis reveals the opposite interspecific relationship between sperm length and the seminal receptacle:sperm ratio than that previously reported, which did not control for phylogenetic and body size effects (Pitnick and Markow 1994a).

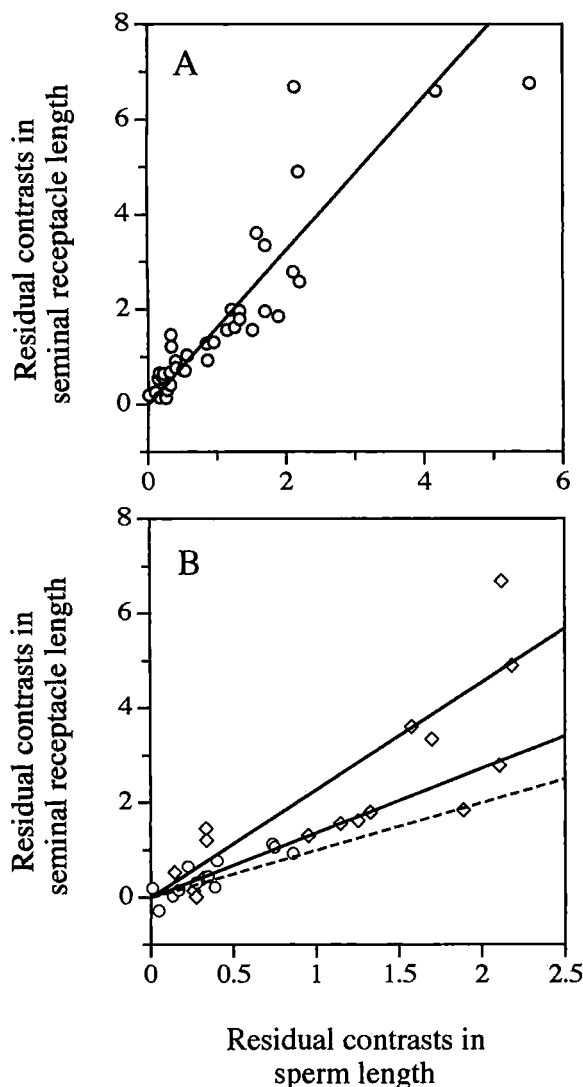We explored whether the pattern of correlated evolution

FIG. 5. The interspecific relationships between residual variation in seminal receptacle length and residual variation in sperm length, after statistically removing body size effects from both variables. Each point is a standardized independent contrast; the RMA regression lines were forced through the origin. (A) Relationship for entire dataset (slope = 1.64). (B) Relationships for seminal receptacle and spermathecae group contrasts (diamonds, slope = 2.29) and seminal receptacle only group contrasts (circles, slope = 1.38). See text for details. Dotted line indicates a slope of 1.0.

between seminal receptacle and sperm length may differ between species that use both the seminal receptacle and the spermathecae for sperm storage (n = 27 species) and those species that use only the seminal receptacle (n = 16 species). To prevent evolutionary changes associated with one pattern of sperm storage from confounding analysis of species exhibiting the alternative sperm storage pattern, it was necessary to restrict each analysis to those contrasts associated with nodes that give rise *only* to species exhibiting the sperm-storage pattern of interest. This process reduced the dataset to 16 contrasts for the seminal-receptacle-and-spermathecae group and 12 contrasts for the seminal-receptacle-only group. After controlling for body size effects, the RMA regressions

of residual contrasts in seminal receptacle length on residual contrasts in sperm length were calculated. The RMA slope for the seminal-receptacle-only group (slope = 1.38) was not significantly different from isometry (i.e., slope = 1.0) (Fig. 5B; t = 1.54, df = 9, P = 0.165). In contrast, the RMA slope for the seminal-receptacle-and-spermathecae group (slope = 2.29) exhibited significant positive allometry (Fig. 5B; t = 2.47, df = 13, P = 0.031). This difference may have important implications for the nature of selection generating the coevolutionary pattern in seminal receptacle and sperm length, as discussed below.

Variation in Spermathecae

In all species for which the spermathecae do not store sperm (n = 40), these organs appear vestigial and are diminutive in size. They are tiny, weakly sclerotized capsules embedded within the thick layer of cells making up the spermathecal envelope. Figures 6A, B, 7A, 8A illustrate the morphology of vestigial spermathecae for four species, each having arisen from an independent evolutionary event. Not surprisingly, these vestigial organs are morphologically distinct from those of even closely related species that have retained their function as sperm-storage organs. For example, compare the spermatheca of *D. micromelanica* (Fig. 6B) with that of its close relative *D. melanica* (Fig. 6D). Spermathecae were classified as nonfunctional without clearly being too tiny to be functional as sperm-storage organs in only two species. In each case, no females were found to contain sperm in the spermathecae, despite many sperm being observed within the seminal receptacle. In addition, size of the spermathecal capsules was greatly reduced in each of these species relative to that of closely related species (cf. Fig. 7A with 7B,C,F; cf. 8A with 8B–D).

Of those species that have retained use of the spermathecae, their spermathecal morphology, apart from some slight size and shape variation, tends to not differ to an appreciable extent. They are typically highly sclerotized, and thus inelastic, spherical-to-ovoid capsules with a well-developed introvert, that part of the capsule that telescopes internally and is in contact with the distal end of the spermathecal duct. For example, Figures 6C–F illustrate spermathecae typical of members of four distinct radiations: the *melanogaster* group (Fig. 6C, *D. melanogaster*), the *melanica* group (Fig. 6D, *D. melanica*), the *quinaria* group (Fig. 6E, *D. subpalustris*), and the *virilis* group (Fig. 6F, *D. montana*).

Two striking exceptions to this typical morphology were observed. The first is found among the three species within the nannoptera species group that have lost use of the seminal receptacle for sperm storage. The three closely related species exhibit three of the most divergent and highly modified spermathecae observed in the genus. The spermathecae of *D. pachea* (Fig. 7B) are the largest known for *Drosophila*. The spermathecae of *D. wassermani* (Fig. 7C) have an unusually elaborate introvert with spines surrounding its base and the distal end expanded into three large branching extensions that fill the capsule and around which the sperm become tightly wrapped (Fig. 7D). Finally, *D. acanthoptera* was the only species found to have nonsclerotized spermathecae, which
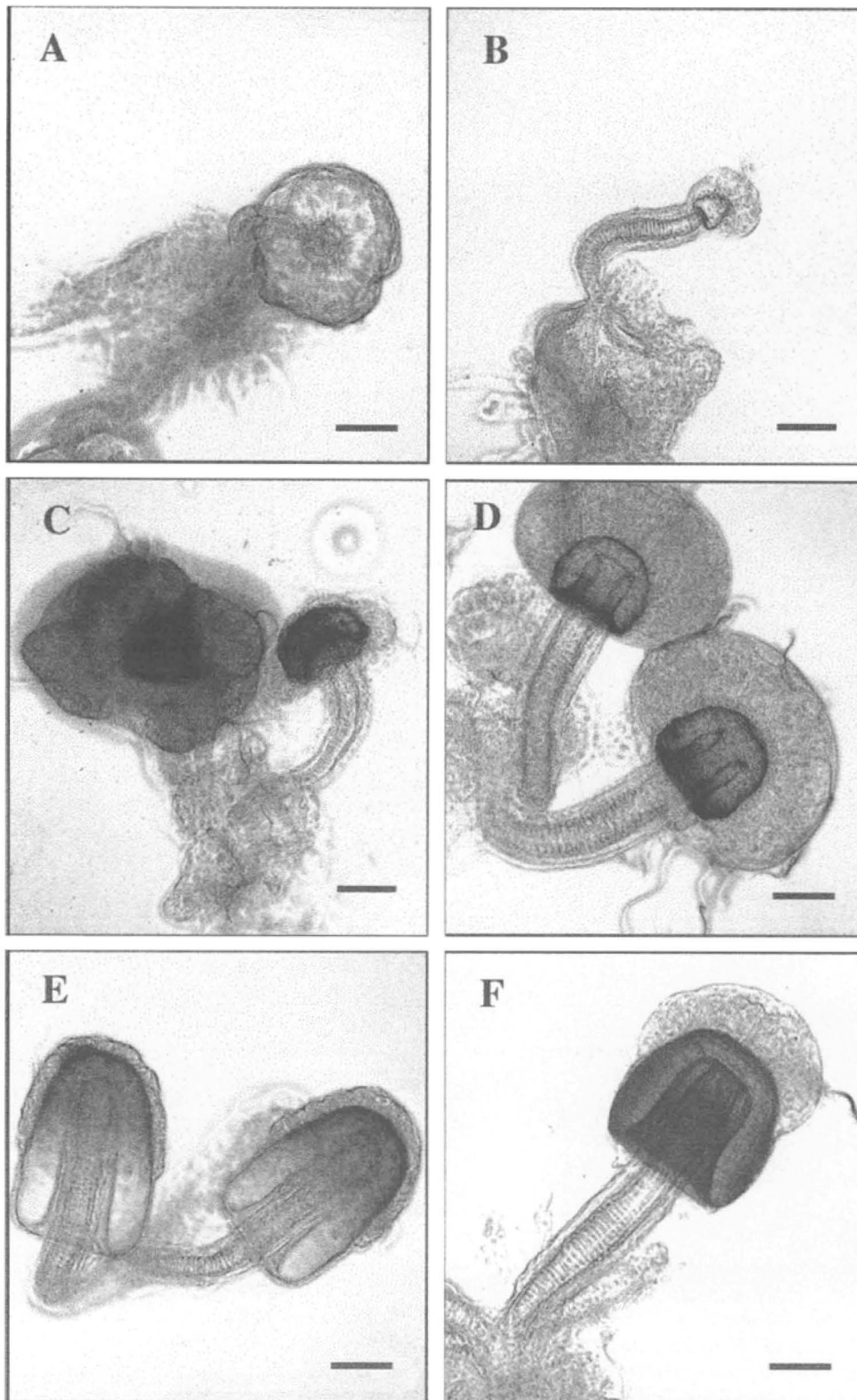
FIG. 6. Photomicrographs of spermathecae illustrating morphological divergence among *Drosophila* species. (A) *D. mojavensis*; (B) *D. micromelanica*; (C) *D. melanogaster*; (D) *D. melanica*; (E) *D. subpalustris*; (F) *D. montana*. All images depicted at the same scale; scale bars = 50 μm.
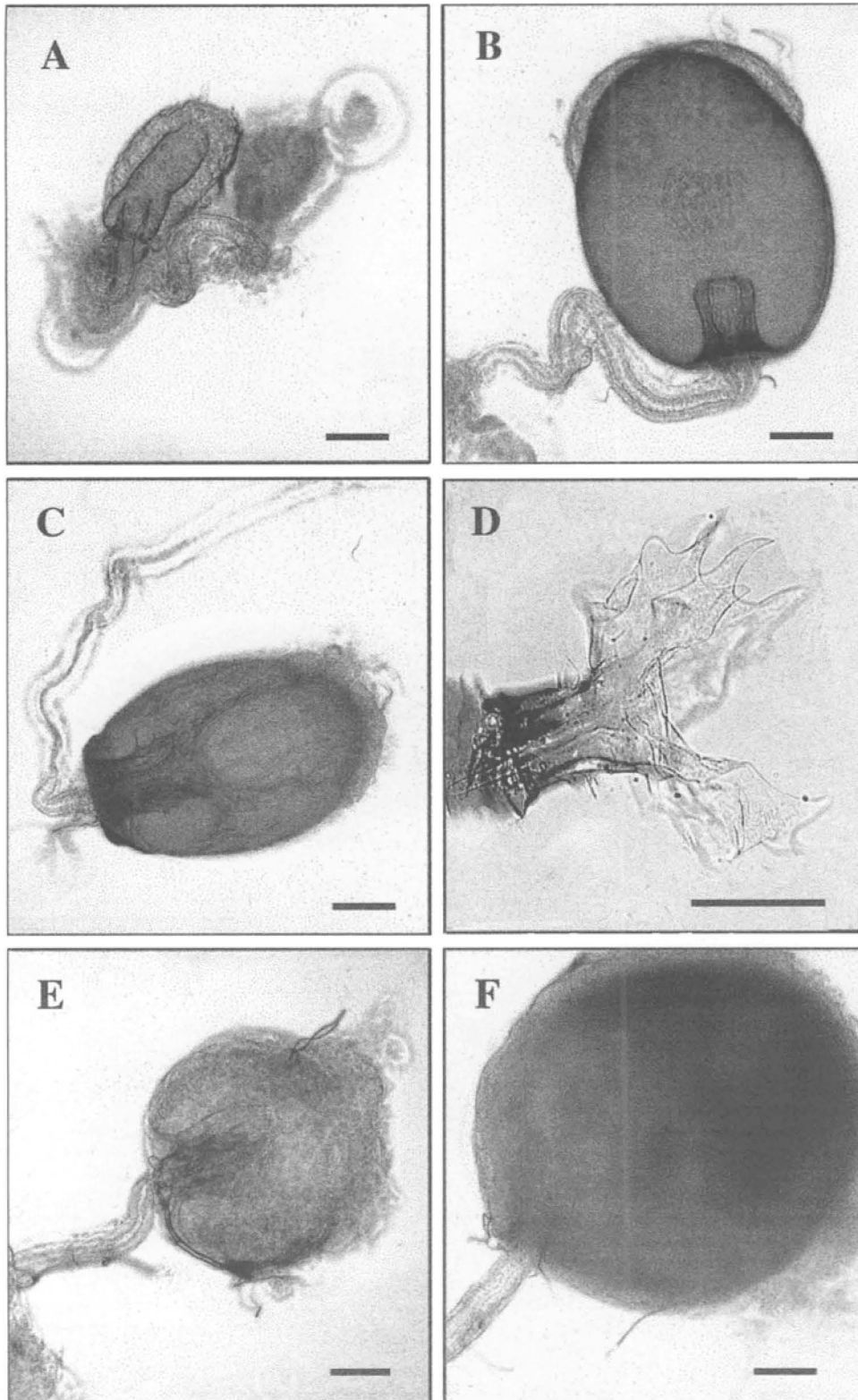
FIG. 7. Photomicrographs of spermathecae illustrating morphological divergence within the *Drosophila nannoptera* species group. (A) *D. nannoptera*; (B) *D. pachea*; (C) *D. wassermani*; (D) *D. wassermani* introvert removed from capsule (magnified 2× relative to C); (E) *D. acanthoptera* (empty); (F) *D. acanthoptera* (filled with sperm). All images (except D) depicted at the same scale; scale bars = 50 μm.
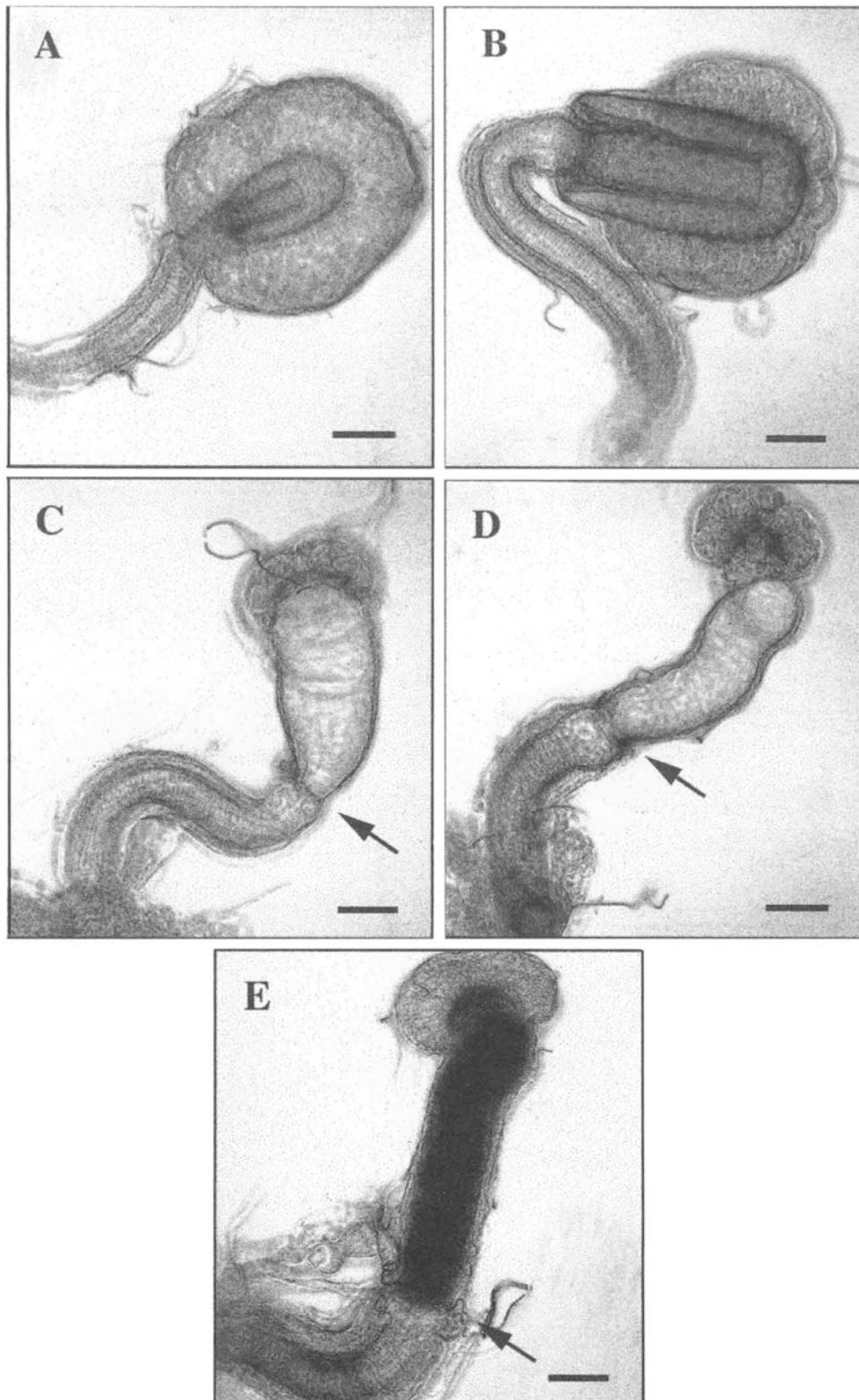
FIG. 8. Photomicrographs of spermathecae illustrating morphological divergence within the *D. hydei* species subgroup. (A) *D. bifurca*; (B) *D. nigrohydei*; (C) *D. eohydei*; (D) *D. hydei* (empty); (E) *D. hydei* (filled with sperm). Arrows indicate sphincter in middle of spermathecal duct. All images depicted at the same scale; scale bars = 50 μm.

are thus elastic and capable of greatly expanding their capacity as sperm are stored (cf. Figs. 7E,F).

The second exception is found within the hydei subgroup of the repleta group. This subgroup is split into two species complexes: the bifurca complex, which includes *D. bifurca* (Fig. 8A), *D. nigrohydei* (Fig. 8B), and two poorly known species, and the hydei complex, which includes *D. eohydei* (Fig. 8C), *D. hydei* (Fig. 8D), and *D. neohydei* (not shown) (Spicer and Pitnick 1996). In all three members of the hydei complex the spermathecal duct has evolved a sphincter at its midpoint (arrows, Figs. 8C–E) and the distal half of the tube has become greatly expanded for sperm storage (compare the spermatheca of *D. hydei* when empty, Fig. 8D, and when full of sperm, Fig. 8E). In these species, the structure homologous with the storage capsule of other species has become reduced to a small disclike cap on the distal end of the storage chamber (Figs. 8C–E).

DISCUSSION

Due to the rapidly divergent nature of female sperm-storage organs within the Drosophilidae and other dipteran families, seminal receptacle and spermathecal morphology has been widely used in systematic studies of these groups (Sturtevant 1925, 1926; Wheeler 1949; Throckmorton 1962). In addition, many preeminent *Drosophila* workers, including M. R. Wheeler (1947, 1954), J. T. Patterson (1947), L. Throckmorton (1962), and W. B. Heed (Russell et al. 1977), have anecdotally described the loss of sperm-storage organ function in various species, mostly in the context of taxonomic descriptions and systematic studies. One goal of the present study is to place these observations into a more cohesive framework for evaluating the pattern and process in the evolution of the females' reproductive tracts.

Evolution of Multiple Kinds of Sperm-Storage Organs in Drosophila

Females of many species possess multiple sperm-storage organs. For example, most flies have three spermathecae (Downes 1968), some mecysmaucheniid spiders have up to 100 spermathecae (Eberhard 1985), and different species of birds have from 500 to 20,000 sperm-storage tubules in their uterovaginal junction (Birkhead and Møller 1992). Possession of multiple kinds of specialized organs for sperm storage occurs more rarely. The ancestral female reproductive tract of lepidopterans includes a corpus bursa, in which the spermatophore is formed during copulation, and a spermatheca, which is simply an expanded portion of the proximal end of the corpus bursa into which the sperm migrate from the spermatophore. In more derived forms, sperm migrate from the corpus bursa to a more expansive bursa (which is perhaps homologous with the ancestral spermatheca), and from there travel to a more specialized spermatheca, which is clearly nonhomologous with the spermatheca of the ancestral form. Because males can access both the bursa and corpus bursa with their genitalia and thus physically interfere with sperm stored there, evolution of the modern spermatheca in lepidopterans may have evolved by selection on females to better control paternity (Common 1970, as discussed in Eberhard 1985, p. 87). In odonates, a similar scenario may explain the origin of spermathecae, their relationship to the bursa copulatrix, and the correlation between intergeneric variation in female sperm-storage organ morphology and the structure of male genitalia (Siva-Jothy 1987; Siva-Jothy and Hooper 1995). The hypothesis that a second type of sperm-storage organ arose in *Drosophila* to prevent direct male access to stored sperm, however, is unsupported. Male genitalia are not able to access any of the sperm-storage organs in any *Drosophila* species or, to the best of our knowledge, in any dipteran.

We assessed the validity of three additional, nonmutually exclusive, hypotheses for the evolution of multiple kinds of sperm-storage organs in *Drosophila* using the comparative data presented here and elsewhere. The first two of these hypotheses contend that multiple organ types result from selection to specialize in more than one function. First, one type of organ may function as a quarantine chamber where sperm are treated to eliminate possible associated pathogens (Birkhead et al. 1993). Second, one organ type may be better suited for long-term sperm storage, whereas the other type is specialized for short-term storage. The third hypothesis contends that multiple kinds of sperm-storage organs are the result of the addition of one or more "improved" types through evolutionary innovation.

Available data refute the first hypothesis and support the second hypothesis. The "quarantine" hypothesis predicts that following insemination sperm should consistently enter one organ type and later be relocated to the other organ type, from which they will be used for fertilization. This prediction is not supported by studies of sperm storage in *D. melanogaster* (Fowler 1973; Gilbert 1981), *D. hydei* (Patterson 1954; S. Pitnick, unpubl. data), *D. pseudoobscura* (Patterson 1954; Snook et al. 1994), or in eight other species (Patterson 1954), which have found that during or shortly following insemination the sperm simultaneously enter into both the spermathecae and the seminal receptacle.

The "differential storage time" hypothesis generates two predictions, both of which must be met: (1) the differing morphologies should reflect adaptation for short- and long-term storage; and (2) because egg laying proceeds following insemination, the pattern of sperm loss from the different storage organs should reflect that sperm are preferentially used initially from the "short-term" organ. The first prediction is met because the spermathecae of all *Drosophila* species are at least partially surrounded by epithelia which, in *D. melanogaster*, have been shown to secrete large amounts of fluid into the lumen of the spermathecal capsule as sperm storage begins (Filosi and Perotti 1975; for weevils also see Villavaso 1975). These secretions may be critical for maintaining sperm viability beyond two to four days postmating, as this is the period of shortened fertility for females without spermathecae (Anderson 1945; Boulétreau-Merle 1977). In contrast, the seminal receptacle has no secretory structure (Miller 1950; Blaney 1970). The second prediction is also generally supported. In the only species for which these kind of data are available: *D. melanogaster* (Nonidez 1920; Gilbert 1981), *D. pseudoobscura* (data for fertilizing sperm morph; Snook et al. 1994), and *D. persimilis* (data for fertilizing sperm morph; Snook 1995), sperm disappear first from the seminal receptacle and are thus presumably used first in fer-

tilization and later from the spermathecae after sperm numbers in the seminal receptacle have substantially declined. The differential storage time hypothesis is thus supported.

The third hypothesis, that the occurrence of multiple kinds of sperm-storage organs results from the origin of a new organ type that functions better than the ancestral type, generates two predictions: (1) where organ replacement rather than coexistence is observed, the ancestral organ type will be lost; and (2) where both organ types are functional, the more derived organ type will be the primary sperm-storage organ. Both of these predictions are supported by this study.

First, spermathecae are present in all Diptera and, in fact, in all insects except where they are known to have been secondarily lost (e.g., bedbugs with traumatic insemination, Carayon 1966). The seminal receptacle, being found only in certain acalyptrate families within the Diptera (Sturtevant 1925, 1926), is therefore clearly the derived organ type. Among acalyptrate families it varies in form from a simple pocket in the uterine wall to a large and heavily chitinized and telescoped pouch (Sturtevant 1925, 1926). Within the Drosophilidae, the seminal receptacle is always a weakly chitinized, slender tube of variable length (e.g., Fig. 1). Phylogenetic analysis suggests that loss of sperm-storage function has frequently occurred in the spermathecae, the ancestral organ type, with 13 discrete evolutionary events having contributed to this character state in 33.6% (38/113) of species examined here. In all of these species, the spermathecae are vestigial, being tiny and weakly sclerotized. In contrast, we identified seminal receptacle dysfunction in only 2.6% (3/113) of species, all resulting from a single evolutionary event (Fig. 2). Stalk-eyed flies in the family Diopsidae exhibit a similar pattern. Of 13 species from six genera studied, all use the seminal receptacle for sperm storage, whereas the spermathecae have become dysfunctional and degenerative in several species (Presgraves et al. 1999).

Direct evidence to support the second prediction of the "new and improved organ" hypothesis, that the seminal receptacle is the primary storage organ, is difficult to obtain. As mentioned above, the few studies quantifying rates of sperm loss from both organ types have consistently concluded that sperm from the seminal receptacle are first used for fertilization. It is also possible, however, that the seminal receptacle is less efficient and leaks more sperm. One possible design improvement may be that the seminal receptacle offers increased capacity for sperm storage. The available data are not consistent with this prediction. Although the seminal receptacle of *D. melanogaster* can store roughly twice as many sperm as can the two spermathecae combined (Gilbert 1981), the two organ types store equivalent numbers of sperm in *D. affinis* (data for fertilizing-sperm morph; Snook 1995), and the combined spermathecae store approximately 2.5 times more sperm than the seminal receptacle in *D. subobscura* (data for long-sperm morphs only; Bressac and Hauschteck-Jungen 1996) and four times more sperm than the seminal receptacle in *D. pseudoobscura* and *D. persimilis* (data for long-sperm morphs only; Snook et al. 1994; Snook 1995).

However, there is strong indirect evidence to suggest that the seminal receptacle is the primary sperm-storage organ in most *Drosophila* species. Assuming that interspecific divergence in organ morphology is due in large part to selection

for adaptive design, it is reasonable to contend that any asymmetry in divergence reflects the extent to which each organ type contributes to individual fitness and thus serves as a target for selective modification. Two such asymmetries are obvious in our data. First, length of the seminal receptacle (presumably a highly functional design component) has evolved at a remarkable rate, varying among *Drosophila* species by 200 times (Figs. 1, 3). In contrast, the structure of spermathecae, for species where this organ is still functional, varies little among even distantly related species (e.g., Fig. 6; Throckmorton 1962). The second important asymmetry is in the pattern of spermathecal evolution among species with different patterns of sperm storage. It is probably not coincidental that three of the most rapidly divergent and morphologically complex spermathecae in the genus are found in the three closely related species that have lost the use of their seminal receptacles (Fig. 7). This observation suggests that when the spermathecae do become the primary organs of sperm storage, they are subject to rapid evolutionary embellishment and diversification.

Two obvious differences between the seminal receptacle and the spermathecae suggest benefits that females might incur by (predominantly) using the seminal receptacle over the spermathecae. First, the sperm mass within the spermathecae appears tangled and disorganized in most species, whereas within the seminal receptacle the sperm generally appear to be neatly straightened. This organizational difference may facilitate more efficient use of sperm from the seminal receptacle and/or improved female control over paternity. Unfortunately, no studies have examined in detail sperm organization within the different storage organs of any *Drosophila* to confirm whether this impression is accurate. Second, differences in the musculature associated with each organ type also suggest that the seminal receptacle may provide females with more sophisticated physiological control over the processes of sperm storage and fertilization. Female-mediated processes are known to contribute to sperm movement within her reproductive tract (Linley 1981; Linley and Simmons 1981, 1983) and these processes have been demonstrated to be largely under muscular control in some species (Davey 1958; Callahan and Cascio 1963; LeCato and Pienkowski 1973; LaMunyon and Eisner 1993; Bloch Qazi et al. 1998). In some insects, there exists a spermathecal muscle that, by altering the shape of the spermathecal capsule, influences sperm storage and/or the movement of sperm from the spermatheca to the site of fertilization (Villavaso 1975; Rodríguez 1994). Although the spermathecal ducts of *Drosophila* have a layer of longitudinal muscle that may aid the movement of sperm (see Camacho 1989, as discussed for tephritid flies in Rodríguez 1994), the spermathecal capsules have no associated musculature. The seminal receptacle, in contrast, has an outer muscle coat throughout its length exhibiting a pattern of muscle filaments characteristic of insect visceral muscle, which may provide sophisticated control over sperm storage and utilization (Blaney 1970).

It is unclear why the sperm-storage function of the spermathecae has been independently lost in many species and retained in others. In addition to possible weak selection and/or insufficient variation to facilitate modification of the spermathecae to a vestigial design in some species, perhaps this

pattern is explained by a combination of the new and improved organ and the differential storage time hypotheses. That is, females of species that have retained use of the spermathecae may remate infrequently or otherwise uniquely benefit from long-term sperm storage, such that the costs of developing, maintaining, and using the spermathecae are outweighed. Studies of female remating interval and egg fertilization rate have not been conducted on a sufficient number of species to evaluate this hypothesis (Markow 1996). Similarly, the variables responsible for loss of use of the seminal receptacle within the nannoptera species group are unknown.

The evolution of novel sperm-storage organs must be rare. With the exception of the derived form of spermatheca described above for lepidoptera, we know of only one other account of this phenomenon. Comparative studies of cicada suggest that structures corresponding to the spermatheca of other homopterans no longer receive sperm. Instead, sperm are stored in a swelling in the oviduct wall (Boulard 1965, as discussed in Eberhard 1985, p. 92). Our discovery that motile sperm are consistently found within the parovaria of *D. nigricruria* (Fig. 4) may represent an early stage in the origin of another type of sperm-storage organ within the Drosophilidae. A similar observation has been made on only one other species, *D. duncani* (Wheeler 1947; Patterson 1954).

Correlated Evolution of Sperm and Female Sperm-Storage Organs

The genus *Drosophila* is unusual in that its members have undergone dramatic divergence in sperm length, with flagellum lengths varying by more than 180 times (Pitnick et al. 1995b). Even sister species can vary substantially in this trait, and particularly gigantic sperm (i.e., > 10 mm) have independently evolved multiple times (Pitnick et al. 1995a). Studies examining relationships between sperm length and various life-history traits have identified numerous evolutionary trade-offs, indicating that longer sperm are relatively costly for males to manufacture (Pitnick and Markow 1994b; Pitnick et al. 1995a,b).

The selective advantages of producing relatively long sperm are unknown. However, a comparative investigation of sperm/egg interactions refutes as a general explanation the hypothesis that sperm length has diverged among *Drosophila* species due to selection to serve some postfertilization function, such as the provisioning of the zygote (Karr and Pitnick 1996). Consequently, the adaptive significance of sperm length variation is best explained by sperm competition because sperm of a given size have some advantage in the direct competition with other sperm to fertilize ova (Gomendio and Roldan 1991; Briskie and Montgomerie 1992, 1993; Parker 1993; Gage 1994; Radwan 1996; Briskie et al. 1997; LaMunyon and Ward 1998) and/or because females have a preference for sperm of a given length and the capacity to discriminate among competing sperm according to their length (Keller and Reeve 1995). If true, then a better understanding of the evolution of giant sperm may be achieved through analysis of the correlated evolution sperm length and female reproductive morphology, because the female reproductive tract is the environment in which sperm compete.

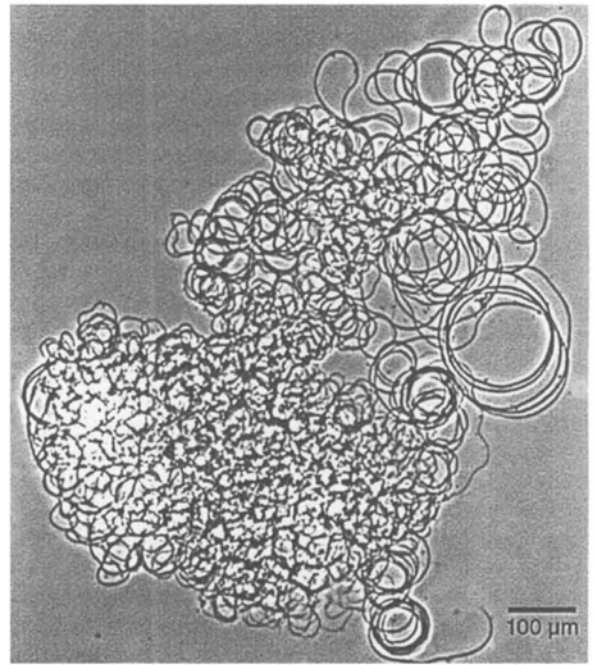A positive interspecific correlation between sperm length

FIG. 9.   Single 58.29-mm-long spermatozoon of *Drosophila bifurca*.

and length of the female sperm-storage organs has been determined for featherwing beetles (Dybas and Dybas 1981), birds (Briskie and Montgomerie 1992, 1993), stalk-eyed flies (Presgraves et al. 1999), and in several previous investigations of *Drosophila* (Hihara and Kurokawa 1987; Pitnick and Markow 1994a; Joly and Bressac 1994). The present study is the first to examine this relationship among *Drosophila* species while statistically controlling for phylogenetic effects and confirms a pattern of strong correlated evolution between these traits.

Four hypotheses may explain the pattern of correlated evolution of sperm and seminal receptacle length in *Drosophila* and other taxa: (1) sequential evolution; (2) sexual conflict; (3) good genes; and (4) runaway selection. The first hypothesis contends that the correlated pattern results from sequential evolution of seminal receptacle length for functional design. That is, sperm length evolves due to selection that is independent of female sperm-storage organ morphology, and female morphological evolution tracks divergence in sperm length due to utilitarian demands of efficient sperm storage, which requires that seminal receptacle length exceeds sperm length.

The sheer magnitude of sperm length in many *Drosophila* species challenges the applicability of this hypothesis. The only location within the female in which sperm are "straightened out" is in the seminal receptacle. For species with very long sperm, therefore, outside of the context of this organ it is difficult to envision how differences in the length of competing males' sperm are "visible" to selection. For example, apart from the seminal receptacle, there is no obvious mechanism by which a 5.83-cm-long sperm (Fig. 9) can provide a fertilization advantage over a 5.80-cm-long sperm in *D. bifurca*, as presumably happens for this costly trait to be maintained (Pitnick et al. 1995a; Pitnick 1996). It seems probable that the giant sperm of *D. bifurca* (Fig. 9) would not be

advantageous to the male in just any female reproductive tract (cf. the two tracts in Fig. 1). Rather, this trait is only adaptive within a female reproductive tract that includes something on the order of an 8.17-cm-long seminal receptacle (Figs. 1, 3). Therefore, if the giant seminal receptacle has been a fundamental part of the selective environment for the evolution of giant sperm in this species, then the evolutionary relationship between them is more interdependent than the sequential evolution hypothesis suggests.

The three remaining hypotheses address the coevolution of male and female traits by postcopulatory sexual selection. The second hypothesis suggests that sperm length and seminal receptacle length have coevolved due to sexual conflict over sperm use (Gowaty 1997; Rice and Holland 1997; Stockley 1997; Holland and Rice 1998), as proposed to explain the correlated evolution of sperm and sperm-storage tubule length in birds (Briskie and Montgomerie 1993). To summarize Briskie and Montgomerie's (1993) model, relatively long sperm-storage tubules are postulated to better promote stratification (layering of ejaculates by pushing some deeper within the organ) of the sperm from successive ejaculates, thereby enhancing a "last in, first out" pattern of sperm precedence. The authors suggested that this pattern benefits females to the extent that they use extrapair copulations to control paternity. Selection on males, at least on the first males to have inseminated females (or previous to last males), favors sperm that are nearly equal in length to the sperm-storage tubule, as this will help prevent their stratification and disuse. The evolutionary consequence of this conflict is a "sexual arms race" with ever lengthening sperm and sperm-storage tubules. The validity of this model cannot yet be determined because the relationship between the ratio of sperm-storage tubule length to sperm length and sperm precedence in birds is unknown, as is the relationship between seminal receptacle length, sperm length, and sperm precedence in *Drosophila*. Alternative means of sexual conflict may also apply to these traits. For example, consistent with the chase-away model of sexual selection (Holland and Rice 1998), longer sperm may benefit males through improved ability to displace or resist displacement by other males' sperm, but at a cost to females. Possible costs include an increase in the number of unfertilized eggs laid due to a suboptimal morphological fit between the seminal receptacle and the longer sperm or a reduction in the number of sperm that can be stored by the female and thus an increase in the need for her to remate more frequently (Partridge and Farquhar 1981; Chapman et al. 1995). Alternatively, consistent with sexual dialectics theory (Gowaty 1997), longer seminal receptacles may represent improved "sperm traps" for postcopulatory discrimination against unfavored males, and longer sperm may be more resistant to being culled by such organs. Sexual conflict has been invoked to explain the correlated evolution of sperm length and female reproductive morphology in stalk-eyed flies (Presgraves et al. 1999).

To the extent that sexual conflict has contributed to the evolution of sperm and seminal receptacle length, it should be noted that females are generally winning the arms race. The relationship between phylogenetically independent contrasts in seminal receptacle length and sperm length is positively allometric (Fig. 5A), meaning that greater evolution-

ary changes in seminal receptacle length are associated with *relatively* smaller changes in sperm length. Therefore, where selection has been most intense, sperm length evolution is not keeping up with seminal receptacle length evolution.

The remaining two hypotheses assume that female sperm-storage organ morphology evolves to promote sperm competition or to select among alternative sperm (Keller and Reeve 1995). Morphology of the female tract therefore represents the proximate basis of (postcopulatory) female sire preference and, in this case, sperm length is the male trait upon which this sire discrimination is based. Giant sperm tails are therefore analogous to showy male traits important in premating sexual selection, such as long tail feathers, antlers, or bright plumage. Either a good genes hypothesis (i.e., parasite resistance or handicap models) or a Fisherian runaway selection hypothesis may explain the evolution of such female preferences (Andersson 1994).

The good genes hypothesis for coevolving seminal receptacle and sperm length predicts that longer seminal receptacles will be favored if they provide a fertilization advantage to longer sperm and if longer sperm are reliably correlated with other heritable aspects of male quality, such as those affecting offspring viability. Because longer sperm are relatively costly to manufacture (Pitnick et al. 1995a; Pitnick 1996), by selectively using longer sperm, females would discriminate against males unable to bear these costs. Alternatively, the runaway selection hypothesis could explain the rapidly divergent and correlated evolution of seminal receptacle and sperm length if there is a functionally assortative relationship between the two traits. Females with longer receptacles would tend to use longer sperm (and vice versa), thus producing both daughters with longer receptacles and sons with longer sperm (or vice versa). This process would create linkage disequilibrium in the population between sperm and seminal receptacle length, which would permit runaway selection of both traits, potentially generating rapid evolution and exaggerated forms of both sperm and seminal receptacles.

Tests of these models and their assumptions, for example, artificially selecting on seminal receptacle length (the female preference) and examining the correlated response in sperm length (the male trait) have not yet been conducted. However, evidence provided here supports the idea that divergence in seminal receptacle length can drive the evolution of sperm length. Our model of morphological compatibility suggests that the seminal receptacle is able to sort or otherwise provide a differential advantage to sperm according to their length, whereas the spermathecae cannot. We therefore predicted that where only the seminal receptacle is used for sperm storage, there should be a one-to-one correspondence between evolutionary change in seminal receptacle length and change in sperm length. In contrast, for species that use both the seminal receptacle and the spermathecae for sperm storage, selection on sperm length will be diluted by the fact that some sperm for fertilizations will come from the spermathecae. This prediction was supported as the macroevolutionary relationship between seminal receptacle length and sperm length was not statistically different from isometry in lineages that only store sperm in the seminal receptacle, yet this relationship exhibited significant positive allometry in lineages that use both

the spermathecae and seminal receptacle for sperm storage (Fig. 5B).

Implications for Reproductive Isolation

To understand how new species come into existence, we need to understand how barriers to interbreeding arise between new species and their ancestors (Rice and Hostert 1993). For any particular case, reproductive isolation is not likely to be due to any one particular factor and premating, postmating/prezygotic, and postzygotic mechanisms may all contribute to the reproductive breakdown (e.g., Moore 1949). However, the vast majority of research on reproductive isolation has focused either on premating or postzygotic isolating mechanisms, with events occurring between insemination and fertilization receiving scant attention (Markow 1997).

Interest in postmating, prezygotic reproductive isolating mechanisms is growing due to recognition of the widespread occurrence of "homogamy." This term refers to a pattern in which, when females are mated to a conspecific and a heterospecific male, the conspecific male tends to sire the majority of offspring, regardless of mating order (Stone and Patterson 1954; Hewitt et al. 1989; Bella et al. 1992; Gregory and Howard 1993, 1994; Robinson et al. 1994; Wade et al. 1994; Albuquerque et al. 1996; Price 1997). The mechanism(s) underlying the conspecific male advantage are generally unknown, but may be attributable in part to the action of seminal fluids (Price 1997). Greater morphological compatibility between the female tract and conspecific sperm due to the evolutionary processes postulated above may also contribute to homogamy.

Within the genus *Drosophila*, both sperm and seminal receptacle morphology are rapidly diverging in a correlated manner. Because the presumptive selection pressure driving this evolution—sexual selection occurring inside of the female reproductive tract—is environment independent, this process is potentially ubiquitous. With restricted gene flow among populations, such divergence may rapidly compromise sperm/female compatibility in among-population crosses. As such, evolution of these traits may constitute an important and widespread source of reproductive isolation.

ACKNOWLEDGMENTS

LITERATURE CITED

Adiyodi, K. G. 1988. Annelida. Pp. 189–250 *in* K. G. Adiyodi and R. G. Adiyodi, eds. Reproductive biology of invertebrates. III. Accessory sex glands. Wiley, Chinchester, U.K.

Albuquerque, G. S., C. A. Tauber, and M. J. Tauber. 1996. Post-mating reproductive isolation between *Chrysopa quadripunctata* and *Chrysopa slonnae*: mechanisms and geographic variation. Evolution 50:1598–1606.

Alexander, R. D., D. C. Marshall, and J. R. Cooley. 1997. Sexual conflict and the evolution of mating systems. Pp. 4–31 *in* J. C. Choe and B. J. Crespi, eds. The evolution of mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge, U.K.

Anderson, R. C. 1945. A study of the factors affecting fertility of lozenge females of *Drosophila melanogaster*. Genetics 30:280–296.

Andersson, M. 1994. Sexual selection. Princeton Univ. Press, Princeton, NJ.

Ashburner, M. 1989. *Drosophila*: a laboratory handbook. Cold Spring Harbor Laboratory Press, New York.

Barrio, E., A. Latorre, and A. Moya. 1994. Phylogeny of the *Drosophila obscura* species group deduced from mitochondrial DNA sequences. J. Mol. Evol. 39:478–488.

Bauer, R. T., and J. W. Martin. 1991. Crustacean sexual biology. Columbia Univ. Press, New York.

Baur, B. 1998. Sperm competition in molluscs. Pp. 255–305 *in* T. R. Birkhead and A. P. Møller, eds. Sperm competition and sexual selection. Academic Press, San Diego, CA.

Bella, J. L., R. K. Butlin, and G. M. Hewitt. 1992. Asymmetrical homogamy and unequal sex ratio from reciprocal mating-order crosses between *Chorthippus parallelus* subspecies. Heredity 68:345–352.

Beverley, S. M., and A. C. Wilson. 1982. Molecular evolution in *Drosophila* and the higher Diptera. I. Micro-complement fixation studies of a larval hemolymph protein. J. Mol. Evol. 18:251–264.

———. 1984. Molecular evolution in *Drosophila* and the higher Diptera. II. A time scale for fly evolution. J. Mol. Evol. 21:1–13.

Birkhead, T. R. 1998. Cryptic female choice: criteria for establishing female sperm choice. Evolution 52:1212–1218.

Birkhead, T. R., and A. P. Møller. 1992. Sperm competition in birds: evolutionary causes and consequences. Academic Press, London.

Birkhead, T. R., A. P. Møller, and W. J. Sutherland. 1993. Why do females make it so difficult for males to fertilize their eggs? J. Theor. Biol. 161:51–60.

Blaney, W. M. 1970. Some observations on the sperm tail of *D. melanogaster*. Dros. Info. Serv. 45:125–127.

Bloch Qazi, M. C., J. R. Aprille, and S. M. Lewis. 1998. Female role in sperm storage in the red flour beetle, *Tribolium castaneum*. Comp. Biochem. Physiol. A 120:641–647.

Boisseau, C., and J. Joly. 1975. Transport and survival of spermatozoa in female amphibia. Pp. 94–104 *in* E. S. E. Hafez and C. G. Thibault, eds. The biology of spermatozoa. Karger, Basel, Switzerland.

Boulétreau-Merle, J. 1977. Rôle des spermathéques dans l'utilisation du sperme et la stimulation de l'ovogenese chez *Drosophila melanogaster*. J. Insect Physiol. 23:1099–1104.

Bressac, C., and E. Hauschteck-Jungen. 1996. *Drosophila subobscura* females preferentially select long sperm for storage and use. J. Insect Physiol. 42:323–328.

Briskie, J. V., and R. Montogomerie. 1992. Sperm size and sperm competition in birds. Proc. R. Soc. Lond. B Biol. Sci. 247:89–95.

———. 1993. Patterns of sperm storage in relation to sperm competition in passerine birds. Condor 95:442–454.

Briskie, J. V., R. Montgomerie, and T. R. Birkhead. 1997. The evolution of sperm size in birds. Evolution 51:937–945.

Brown, W. D., B. J. Crespi, and J. C. Choe. 1997. Sexual conflict and the evolution of mating systems. Pp. 352–377 *in* J. C. Choe and B. J. Crespi. The evolution of mating systems in insects and arachnids. Cambridge Univ. Press, Cambridge, U.K.

Caccone, A., J. M. Gleason, and J. R. Powell. 1992. Complementary DNA-DNA hybridization in *Drosophila*. J. Mol. Evol. 34:130–140.

Callahan, P. S., and T. Cascio. 1963. Histology of the reproductive tracts and transmission of sperm in the corn earworm, *Heliothis zea*. Ann. Entomol. Soc. Am. 56:535–556.

Carayon, J. 1966. Traumatic insemination and the paragenital system. Pp. 81–166 *in* R. Usinger, ed. Monograph of Cimicidae. Thomas Say Foundation 7, Entomological Society of America, Philadelphia, PA.

Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating *Drosophila melanogaster* females is mediated by male accessory gland products. Nature 373:241–244.

Clarke, M. R. B. 1980. The reduced major axis of a bivariate sample. Biometrika 67:441–446.

Cunningham, C. W., K. E. Omland, and T. H. Oakley. 1998. Reconstructing ancestral character states: a critical reappraisal. Trends Ecol. Evol. 13:361–366.

Davey, K. G. 1958. The migration of spermatozoa in the female of *Rhodnius prolixus* Stal. J. Exp. Biol. 30:694–701.

———. 1965. Reproduction in insects. Oliver and Boyd, London.

Davies, N. B. 1989. Sexual conflict and the polygamy threshold. Anim. Behav. 38:226–234.

DeSalle, R. 1992. The phylogenetic relationships of flies in the family Drosophilidae deduced from mtDNA sequences. Mol. Phylog. Evol. 1:31–40.

Donoghue, M. J., and D. D. Ackerly. 1996. Phylogenetic uncertainties and sensitivity analysis in comparative biology. Phil. Trans. R. Soc. Lond. B Biol. Sci. 351:1241–1249.

Downes, J. A. 1968. Notes on the organs of processes of sperm-transfer in the lower Diptera. Can. Entomol. 100:608–617.

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

Eberhard, W. G. 1985. Sexual selection and animal genitalia. Harvard Univ. Press, Cambridge, MA.

———. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, NJ.

Felsenstein, J. 1985. Phylogenies and the comparative method. Am. Nat. 125:1–15.

Filosi, M., and M. E. Perotti. 1975. Fine structure of the spermatheca of *Drosophila melanogaster*. J. Submicr. Cytol. 7:259–270.

Fowler, G. L. 1973. Some aspects of the reproductive biology of *Drosophila*: sperm transfer, sperm storage, and sperm utilization. Adv. Genet. 17:293–360.

Gack, C., and K. Peschke. 1994. Spermathecal morphology, sperm transfer and a novel mechanism of sperm displacement in the rove beetle, *Aleochara curtula* (Coleoptera, Staphylinidae). Zoomorphology 114:227–237.

Gage, M. J. G. 1994. Associations between body size, mating pattern, testis size, and sperm lengths across butterflies. Proc. R. Soc. Lond. B Biol. Sci. 258:247–254.

Garland, T. 1985. Ontogenetic and individual variation in size, shape and speed in the Australian agamid lizard *Amphibolurus nuchalis*. J. Zool. 207:425–439.

Garland, T., P. H. Harvey, and A. R. Ives. 1992. Procedures for the analysis of comparative data using phylogenetically independent contrasts. Syst. Biol. 41:18–32.

Garland, T., A. W. Dickerman, C. M. Janis, and J. A. Jones. 1993. Phylogenetic analysis of covariance by computer simulation. Syst. Biol. 42:265–292.

Gilbert, D. G. 1981. Ejaculate esterase-6 and initial sperm use by female *Drosophila melanogaster*. J. Insect Physiol. 27:641–650.

Gomendio, M., and E. R. S. Roldan. 1991. Sperm competition influences sperm size in mammals. Proc. R. Soc. Lond. B Biol. Sci. 243:181–185.

Gowaty, P. A. 1997. Sexual dialectics, sexual selection, and variation in reproductive behavior. Pp. 351–384 *in* P. A. Gowaty, ed. Feminism and evolutionary biology. Chapman and Hall, New York.

Gowaty, P. A., and N. Buschhaus. 1998. Ultimate causation of aggressive and forced copulation in birds: female resistance, the CODE hypothesis, and social monogamy. Am. Zool. 38:207–225.

Gregory, P. G., and D. J. Howard. 1993. Laboratory hybridization studies of *Allonemobius fasciatus* and *A. socius* (Orthoptera: Gryllidae). Ann. Entomol. Soc. Am. 86:694–701.

———. 1994. A postinsemination barrier to fertilization isolates two closely related ground crickets. Evolution 48:705–710.

Grimaldi, D., A. C. James, and J. Jaenike. 1992. Systematics and modes of reproductive isolation in the holarctic *Drosophila testacea* species group (Diptera: Drosophildae). Ann. Entomol. Soc. Am. 85:671–685.

Harvey, P. H., and M. D. Pagel. 1991. The comparative method in evolutionary biology. Oxford Univ. Press, Oxford, U.K.

Hellriegel, B., and P. I. Ward. 1998. Complex female reproductive tract morphology: its possible use in postcopulatory female choice. J. Theor. Biol. 190:179–186.

Hewitt, G. M., P. Mason, and R. A. Nichols. 1989. Sperm precedence and homogamy across a hybrid zone in the alpine grasshopper *Podisma pedestris*. Heredity 62:343–353.

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

Holland, B., and W. R. Rice. 1998. Chase-away sexual selection: antagonistic seduction versus resistance. Evolution 52:1–7.

Jefferson, M. C. 1977. Breeding biology of *Drosophila pachea* and its relatives. Ph.D. diss., University of Arizona, Tucson, AZ.

Joly, D., and C. Bressac. 1994. Sperm length in Drosophildae (Diptera): estimation by testis and receptacle lengths. Int. J. Insect Morphol. Embryol. 23:85–92.

Kadow, P. E. 1954. An analysis of sexual behavior and reproductive physiology in the guppy, *Lebistes reticulatus* (Peters). Ph.D. diss., New York University, New York.

Karr, T. L., and S. Pitnick. 1996. The ins and outs of fertilization. Nature 379:405–406.

Keller, L., and H. Reeve. 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. Adv. Study Behav. 24:291–315.

Knowlton, N., and S. R. Greenwell. 1984. Male sperm competition avoidance mechanisms: The influence of female interests. Pp. 61–84 *in* R. L. Smith, ed. Sperm competition and the evolution of animal mating systems. Academic Press, New York.

Kwiatowski, J., D. Skarecky, K. Bailey, and F. J. Ayala. 1994. Phylogeny of *Drosophila* and related genera inferred from the nucleotide sequence of the Cu, Zn *Sod* gene. J. Mol. Evol. 38:443–454.

LaMunyon, C. W., and T. Eisner. 1993. Postcopulatory sexual selection in an arctiid moth (*Utetheisa ornatrix*). Proc. Natl. Acad. Sci. USA 90:4689–4692.

LaMunyon, C., and S. Ward. 1998. Larger sperm outcompete smaller sperm in the nematode *C. elegans*. Proc. R. Soc. Lond. B Biol. Sci. 265:1–6.

LeCato, G. L., and R. L. Pienkowski. 1973. Insemination and sperm storage in the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae). Int. J. Insect Morphol. Embryol. 2:257–263.

Linley, J. R. 1981. Emptying of the spermatophore and spermathecal filling in *Culicoides melleus* (Coq.)(Diptera: Ceratopogonidae). Can. J. Zool. 59:347–356.

Linley, J. R., and K. R. Simmons. 1981. Sperm motility and spermathecal filling in lower Diptera. Int. J. Invert. Repro. 4:137–146.

———. 1983. Quantitative aspects of sperm transfer in *Simulium decorum* (Diptera: Simuliidae). J. Insect Physiol. 9:581–584.

Maddison, D. R. 1994. Phylogenetic methods for inferring the evolutionary history and processes of change in discretely valued characters. Annu. Rev. Entomol. 39:267–292.

Maddison, W. P., and D. R. Maddison. 1997. MacClade: analysis of phylogeny and character evolution. Vers. 3.05. Sinauer, Sunderland, MA.

Markow, T. A. 1996. Evolution of *Drosophila* mating systems. Pp. 73–106 *in* M. K. Hecht, ed. Evolutionary biology. Vol. 29. Plenum Press, New York.

———. 1997. Assortative fertilization in *Drosophila*. Proc. Natl. Acad. Sci. USA 94:7756–7760.

Martins, E. P., and T. Garland. 1991. Phylogenetic analyses of the correlated evolution of continuous characters: a simulation study. Evolution 45:534–557.

Miller, A. 1950. The internal anatomy and histology of the imago

of *Drosophila melanogaster*. Pp. 420–534 *in* M. Demerec, ed. Biology of *Drosophila*. Hafner, New York.

Moore, J. A. 1949. Patterns of evolution in the genus *Rana*. Pp. 315–338 *in* G. L. Jepsen, E. Mayr and G. G. Simpson, eds. Genetics, paleontology, and evolution. Princeton Univ. Press, Princeton, NJ.

Nonidez, J. F. 1920. The internal phenomena of reproduction in *Drosophila*. Biol. Bull. 39:207–230.

Olsson, M., and T. Madsen. 1998. Sexual selection and sperm competition in reptiles. Pp. 503–577 *in* T. R. Birkhead and A. P. Møller, eds. Sperm competition and sexual selection. Academic Press, San Diego, CA.

Omland, K. E. 1997. Examining two standard assumptions of ancestral reconstructions: repeated loss of dichromatism in dabbling ducks (Anatini). Evolution 51:1636–1646.

Otronen, M., P. Reguera, and P. I. Ward. 1997. Sperm storage in the yellow dung fly *Scathophaga stercoraria*: identifying the sperm of competing males in separate female spermathecae. Ethnology 103:844–854.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

———. 1984. Sperm competition and the evolution of animal mating strategies. Pp. 1–60 *in* R. L. Smith, ed. Sperm competition and the evolution of animal mating systems. Academic Press, New York.

———. 1993. Sperm competition games: Sperm size and sperm number under adult control. Proc. R. Soc. Lond. B Biol. Sci. 253:245–254.

Partridge, L., and M. Farquhar. 1981. Sexual activity reduces lifespan of male fruitflies. Nature 294:580–582.

Patterson, J. T. 1943. Studies in the genetics of *Drosophila*. III. The Drosophilidae of the southwest. Univ. Texas Publ. 4313:7–203.

———. 1947. II. Sexual isolation in the mulleri subgroup. Univ. Texas Publ. 4720:32–40.

———. 1954. Fate of the sperm in the reproductive tract of the *Drosophila* female in homogamic matings. Univ. Texas Publ. 5422:19–37.

Pelandakis, M., and M. Solignac. 1993. Molecular phylogeny of *Drosophila* based on ribosomal RNA sequences. J. Mol. Evol. 37:525–543.

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

Pitnick, S., and T. A. Markow. 1994a. Male gametic strategies: Sperm size, testes size, and the allocation of ejaculate among successive mates by the sperm-limited fly *Drosophila pachea* and its relatives. Am. Nat. 143:785–819.

———. 1994b. Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. Proc. Natl. Acad. Sci. USA 91:9277–9281.

Pitnick, S., T. A. Markow, and M. F. Riedy. 1991. Transfer of ejaculate and incorporation of male-derived substances by females in the nannoptera species group (Diptera: Drosophilidae). Evolution 45:774–780.

Pitnick, S., T. A. Markow, and G. S. Spicer. 1995a. Delayed male maturity is a cost of producing large sperm in *Drosophila*. Proc. Natl. Acad. Sci. USA 92:10614–10618.

Pitnick, S., G. S. Spicer, and T. A. Markow. 1995b. How long is a giant sperm? Nature 375:109.

———. 1997. Phylogenetic examination of female incorporation of ejaculate in *Drosophila*. Evolution 51:833–845.

Polak, M. 1993. Parasites increase fluctuating asymmetry of male *Drosophila nigrospiracula*: implications for sexual selection. Genetica 89:255–265.

Powell, J. R. 1997. Progress and prospects in evolutionary biology: the *Drosophila* model. Oxford Univ. Press, New York.

Powell, J. R., and R. DeSalle. 1995. *Drosophila* molecular phylogenies and their uses. Evol. Biol. 28:87–138.

Presgraves, D. C., R. H. Baker, and G. S. Wilkinson. 1999. Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. Proc. R. Soc. Lond. B Biol. Sci. 266:1041–1047.

Price, C. S. C. 1997. Conspecific sperm precedence in *Drosophila*. Nature 388:663–666.

Racey, P. A. 1979. The prolonged storage and survival of spermatozoa in Chiroptera. J. Repro. Fert. 56:391–402.

Radwan, J. 1996. Intraspecific variation in sperm competition success in the bulb mite: a role of sperm size. Proc. R. Soc. Lond. B Biol. Sci. 263:855–859.

Ree, R. H., and M. J. Donoghue. 1998. Step matrices and the interpretation of homoplasy. Syst. Biol. 47:582–588.

Rice, W. R. 1996. Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. Nature 381:232–234.

———. 1997. Intergenomic conflict, interlocus antagonistic evolution, and the evolution of reproductive isolation. Pp. 261–270 *in* D. J. Howard and S. H. Berlocher, eds. Endless forms: species and speciation. Oxford Univ. Press, Oxford, U.K.

Rice, W. R., and B. Holland. 1997. The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. Behav. Ecol. Sociobiol. 41:1–10.

Rice, W. R., and E. E. Hostert. 1993. Laboratory experiments on speciation: what have we learned in 40 years? Evolution 47:1637–1653.

Robinson, T., N. A. Johnson, and M. J. Wade. 1994. Postcopulatory, prezygotic isolation: intraspecific and interspecific sperm precedence in *Tribolium* spp. flour beetles. Heredity 73:155–159.

Rodríguez, V. 1994. Function of the spermathecal muscle in *Chelymorpha alternans* Boheman (Coleoptera: Chrysomelidae: Cassidinae). Physiol. Entomol. 19:198–202.

Rowe, L., G. Arnqvist, A. Sih, and J. J. Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends Ecol. Evol. 9:289–293.

Russell, J. S., B. L. Ward, and W. B. Heed. 1977. Sperm storage and hybridization in *D. nannoptera* and related species. Dros. Info. Serv. 52:70.

Russo, C. A. M., N. Takezaki, and M. Nei. 1995. Molecular phylogeny and divergence times of Drosophilid species. Mol. Biol. Evol. 12:391–404.

Shugart, G. W. 1988. Uterovaginal sperm-storage glands in sixteen species with comments on morphological differences. Auk 105:379–385.

Siva-Jothy, M. T. 1987. The structure and function of the female sperm-storage organs in libellulid dragonflies. J. Insect Physiol. 33:559–567.

Siva-Jothy, M. T., and R. E. Hooper. 1995. The disposition and genetic diversity of stored sperm in females of the damselfly *Calopteryx splendens xanthostoma* (Charpentier). Proc. R. Soc. Lond. B Biol. Sci. 259:313–318.

Smuts, B. B., and R. W. Smuts. 1993. Male aggression and sexual coercion of females in nonhuman primates and other mammals: evidence and theoretical implications. Adv. Study Behav. 22:1–63.

Snodgrass, R. E. 1935. The principles of insect morphology. McGraw Hill, New York.

Snook, R. R. 1995. The evolution of sperm polymorphism in the *Drosophila obscura* group, Ph.D. diss. Arizona State University, Tempe, AZ.

Snook, R. R., T. A. Markow, and T. L. Karr. 1994. Functional nonequivalence of sperm in *Drosophila psuedoobscura*. Proc. Natl. Acad. Sci. USA 91:11222–11226.

Spicer, G. 1988. Molecular evolution among some *Drosophila* species groups as indicated by two-dimensional electrophoresis. J. Mol. Evol. 27:250–260.

———. 1991. Molecular evolution and phylogeny of the *Drosophila virilis* species group as inferred by two-dimensional electrophoresis. J. Mol. Evol. 1991:379–394.

———. 1992. Reevaluation of the phylogeny of the *Drosophila virilis* species group (Diptera: Drosophilidae). Ann. Entomol. Soc. Am. 85:11–25.

Spicer, G. S., and J. Jaenike. 1996. Phylogenetic analysis of breeding site use and a-amanitin tolerance within the *Drosophila quinaria* species group. Evolution 50:2328–2337.

Spicer, G. S., and S. Pitnick. 1996. Molecular systematics of the *Drosophila hydei* subgroup as inferred from mitochondrial DNA sequences. J. Mol. Evol. 43:281–286.

Stockley, P. 1997. Sexual conflict resulting from adaptations to sperm competition. Trends Ecol. Evol. 12:154–159.

Stone, W. S., and J. T. Patterson. 1954. III. Fertilizations in multiple matings between species of the virilis group. Univ. Texas Publ. 5422:38–45.

Sturtevant, A. H. 1925. The seminal receptacles and accessory glands of the Diptera, with special reference to the Acalypterae. J. New York Entomol. Soc. 33:195–215.

———. 1926. The seminal receptacles and accessory glands of the Diptera, with special reference to the Acalypterae. J. New York Entomol. Soc. 34:1–22.

Sullivan, D. T., P. W. Atkinson, and W. T. Starmer. 1990. Molecular evolution of the alcohol dehydrogenase genes in the genus *Drosophila*. Evol. Biol. 24:107–147.

Swofford, D. L., and W. P. Maddison. 1987. Reconstructing ancestral characters state under wagner parsimony. Math. Biosci. 87:119–199.

Thomas, R. H., and D. W. Zeh. 1984. Sperm transfer and utilization strategies in arachnids: ecological and morphological constraints. Pp. 179–221 *in* R. L. Smith, ed. Sperm competition and the evolution of animal mating systems. Academic Press, New York.

Throckmorton, L. H. 1962. The problem of phylogeny in the genus *Drosophila*. Univ. Texas Publ. 6205:207–343.

———. 1975. The phylogeny, ecology and geography of *Drosophila*. Pp. 421–469 *in* R. C. King, ed. Handbook of genetics. Vol. 3. Plenum Press, New York.

Villavaso, E. J. 1975. The role of the spermathecal gland of the boll weevil, *Anthonomus grandis*. J. Insect Physiol. 21:1457–1462.

Wade, M. J., H. Patterson, N. W. Chang, and N. A. Johnson. 1994. Postcopulatory, prezygotic isolation in flour beetles. Heredity 72:163–167.

Walker, W. F. 1980. Sperm utilization strategies in nonsocial insects. Am. Nat. 115:780–799.

Ward, P. I. 1993. Females influence sperm storage and use in the yellow dung fly *Scathophaga stercoraria* (L.). Behav. Ecol. Sociobiol. 32:313–319.

———. 1998. A possible explanation for cryptic female choice in the yellow dung fly, *Scathophaga stercoraria* (L.). Ethol. 104:97–110.

Wasserman, M. 1962. Cytological studies of the repleta group of the genus *Drosophila*: V. The mulleri subgroup. Univ. Texas Publ. 6205:85–117.

———. 1992. Cytological evolution of the *Drosophila repleta* species group. Pp. 455–552 *in* C. B. Krimbas and J. R. Powell, eds. *Drosophila* inversion polymorphism. CRC Press, Boca Raton, FL.

Wheeler, M. R. 1947. IV. The insemination reaction in intraspecific matings of *Drosophila*. Univ. Texas Publ. 4720:78–115.

———. 1949. Taxonomic studies on the Drophilidae. Univ. Texas Publ. 4920:157–195.

———. 1954. Taxonomic studies on American *Drosophila*. Univ. Texas Publ. 4720:47–64.

Wigglesworth, V. B. 1965. The principles of insect physiology. Methuen, London.

Acting Editor: P. Gowaty