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The long and short of sperm polymorphisms in insects

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ABSTRACT

Production of more than one morphological type of sperm in a common testis has been documented for a variety of invertebrates, including gastropods, spiders, centipedes, and insects. This unusual phenomenon is difficult to explain by current theory, particularly since available evidence indicates that one sperm type is often incapable of effecting fertilization. In this review we critically examine evidence on the distribution and development of sperm heteromorphisms among insects in light of competing hypotheses for the evolutionary origin, maintenance, and function of a non-fertilizing class of sperm. To date, no single hypothesis, including alternatives which assume non-fertilizing sperm are non-adaptive, or that they provision, facilitate, or compete with fertilizing sperm, has received strong empirical support by any group of insects. The diversity of sperm heteromorphisms suggests that non-fertilizing sperm may have different functions in different clades or even serve multiple functions within a clade. We suggest that insight could be gained from (1) new models for the evolution of sperm polymorphism, (2) comparative studies that focus on multiple traits simultaneously (e.g. sperm number, proportion, length, and remating rate) and utilize clades in which more than one gain or loss of sperm heteromorphism has been documented (e.g. Pentatomidae, Carabidae, or Diopsidae), and (3) experimental studies that exploit individual variation or directly manipulate the composition of the male ejaculate.

Key words: Insecta, sperm heteromorphism, polymegaly, apyrene, eupyrene, heteroploidy.

CONTENTS

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I. INTRODUCTION

The potential for post-copulatory competition between, and therefore selection on, ejaculates of different males was first pointed out by Parker (1970). Sperm competition is now widely recognized as a powerful form of sexual selection (Birkhead & Møller, 1998). Models of sperm competition, such as those that assume a fair raffle, have been used to predict the optimal size and number of sperm in an ejaculate (e.g. Parker, 1982). However, sperm morphology, not unlike secondary sexual characters, can evolve rapidly (Baccetti & Afzelius, 1976; Jamieson, 1987; Joly *et al*., 1989) and displays spectacular diversity, particularly among species with internal fertilization. Sperm polymorphism, the production of two or more discrete morphological classes of spermatozoa within a single male ejaculate, represents a curious exception to the typical pattern of many tiny sperm and is not easily explained by simple models of sperm competition.

The regular production of multiple classes of sperm was first reported in prosobranch gastropods in 1836 (Seibold, 1836; see Hodgson, 1997 for a historical review of sperm polymorphism in gastropods). Heteromorphic sperm have since been described for a wide variety of other invertebrates, apparently evolving independently several times in arthropods, e.g. spiders (Rosati, Baccetti & Dallai, 1970), centipedes (Jamieson, 1986), and insects (Sivinski, 1980). In this review we limit our discussion to the class Insecta, a taxonomic group that displays a wide variety of sperm polymorphisms. For example, all moths and butterflies produce both nucleated and anucleated sperm while some bug and beetle species produce sperm which vary in chromosome complement. Sperm morphs also differ in size in some bugs and beetles, in length in some flies, or in shape in some wasps.

The first system for the classification of invertebrate sperm used chromosome complement to distinguish sperm types (Meves, 1903). Sperm with a haploid complement of chromosomes were termed eupyrene; those with chromosome numbers in excess of a haploid complement were called hyperpyrene while those with less were called oligopyrene. Apyrene sperm contain no chromatin. Early on it was recognized that many of these alternative sperm morphs, particularly those that deviated in chromosome content, would not be fertilization competent and were therefore labeled 'atypical'. Because of the connotations of words such as 'atypical', Healy and

Jamieson (1981), among others, have suggested that non-fertilizing sperm should be named paraspermatozoa and fertilizing sperm called euspermatozoa. Thus, non-fertilizing sperm, even those with normal chromatin content, are parasperm. We use these terms throughout the remainder of this paper.

What evolutionary forces favour the production and maintenance of an infertile class of sperm? The answer to this question has remained elusive, in part, because individuals working on different groups have not always considered the same alternative hypotheses. Below, we first discuss the hypotheses that have been proposed for the function of parasperm. Then we discuss the nature of heteromorphic sperm in Lepidoptera, Diptera, Hemiptera, Hymenoptera, Coleoptera and a few other isolated cases. We end by discussing how theory, comparative analyses, and future experiments can shed light on the forces responsible for the recurrent evolution of polymorphic sperm.

II. HYPOTHESES FOR THE FUNCTION OF HETEROMORPHIC SPERM

The possible functions of heteromorphic sperm in insects were first reviewed by Sivinski (1980), while Silberglied, Shepherd & Dickinson (1984) discussed the possible role of apyrene sperm in the Lepidoptera. To emphasize the putative role of selection here, we group prior hypotheses concerning the possible function of heteromorphic sperm into four categories: (1) non-adaptive, (2) provisioning, (3) facilitation, and (4) competition (Table 1).

(1) Non-adaptive

Most early researchers generally regarded parasperm as functionless aberrations, products of a hermaphroditic tendency, an abnormal physiological environment during development, or the consequence of replication error during meiosis (see references in Woodward, 1940). As such, sperm heteromorphism was considered non- or even maladaptive because resources were being wasted on non-fertilizing gametes (e.g. Goldschmidt, 1916).

Cohen (1973), a proponent of the replication error hypothesis, proposed that 'atypical' sperm persist because the cost of selectively eliminating them would be greater than the resultant loss of resources. Although this argument remains credible with regard to mammalian sperm that deviate from normal morphology (e.g. Cohen, 1973; Harcourt, 1989, 1991), it appears much less tenable, for a variety of reasons, with respect to most insect sperm polymorphisms. First, some insects produce such a large number of the alternative sperm morph, often in excess of the fertilizing sperm morph, that 'it involves a waste that goes beyond the ordinary, its elimination by natural selection might well be

Table 1. *Hypotheses concerning the function of parasperm*

Category	Hypothesis	Function	References
Non-adaptive	Non-adaptive	None	Goldschmidt (1916); Cohen (1973)
Facilitation	Transportation	Aid in emigration from testes	Katsuno (1977); Riemann *et al.* (1974); Osanai *et al.* (1987)
		Aid in migration within female	Iriki (1941); Holt & North (1970*a*); Friedlander & Gitay, 1972
	Capacitation	Initiate capacitation	Osanai *et al.* (1987)
Provisioning	Provisioning	Provide nutrients to female	Riemann & Gassner (1973)
		Provide nutrients to eusperm	Riemann & Gassner (1973); Sivinski (1980)
		Provide nutrients to ovum	Sivinski (1980); Schrader (1960*b*); Richards (1963)
Competition	Elimination	Remove/flush stored sperm	Silberglied *et al.* (1984)
		Disable stored sperm	Silberglied *et al.* (1984); Baker & Bellis (1988, 1989)
	Cheap filler	Delay remating	Silberglied *et al.* (1984)
	Blocking	Block sperm entry	Baker & Bellis (1988, 1989)
	Differential success	Both fertilization competent	Sivinski (1980); Joly (1987); Joly *et al.* (1989, 1991)
Other	Sex ratio	Alter sex ratio	Lee & Wilkes (1965); Wilkes & Lee, 1965

expected (Schrader, 1960*b*)'. More significantly, sperm-polymorphic insects produce distinct morphological (and functional) classes of sperm, where individual variation between sperm within a morph is much less than the variation between morphs. The developmental patterns and structure of these morphological variants appears to be constant in all species studied to date (e.g. Friedlander, 1997; Pasini *et al*., 1996; Schrader, 1960*a*). Thus, the developmental constancy of insect parasperm differs from mammalian 'atypical' sperm, which represent an amalgam of deviant sperm morphologies (for review see Harcourt, 1991).

(2) Provisioning

Subsequent hypotheses assumed an adaptive, functional role for parasperm. A nutritive role for parasperm is the longest standing of these (references as early as 1896 cited in Giusti & Selmi, 1982). Parasperm that degenerate in the female reproductive tract could provide a source of energy (e.g. polysaccharides, proteins, nucleic acids) either to fertile sperm, the female, or the developing zygote, thus providing a fitness advantage to the female mate or offspring (see Riemann & Gassner, 1973; Sivinski, 1980; Snook & Markow, 1996). Supernumerary sperm feeding the ova has also been proposed as a possible function for paraspermatozoa in stinkbugs (Schrader, 1960*b*) and cockroaches (Richards, 1963); both of which produce large, nutrient-rich gametes. Under the polyspermy scenario (more than one sperm entering the ovum), the male would be more assured that his offspring were receiving the nutritional benefit provided by his nuptial gift. While the prevalence of polyspermy in insects is unknown, it appears to occur with some frequency in some insects (e.g. *Drosophila obscura* group: Snook, 1998*b*).

(3) Facilitation

(*a*) *Transportation*

The first set of hypotheses in this category proposed that parasperm function as an aid to transport longer sperm from the site of production in the male testes to the site of fertilization in the female reproductive tract. Based on the observations in Lepidoptera that, in most cases, apyrene sperm migrate out of the testes before eupyrene sperm (Katsuno, 1977; Riemann, Thorson & Ruud, 1974), Katsuno (1977) hypothesized that apyrene sperm perforate the testicular basement membrane and thereby facilitate emigration of eupyrene sperm bundles from the testes to the efferent ducts.

In an array of Lepidopteran species, apyrene sperm become vigorously motile upon ejaculation (Shepard, 1974); eupyrene sperm acquire a much lower motility, if they acquire motility at all, only after insemination (Etman & Hooper, 1979*a*; Holt & North, 1970*a*; Iriki, 1941). This observation has led a variety of researchers to suggest that apyrene sperm somehow physically propel or assist the movement of eupyrene sperm within the female reproductive tract (Friedlander & Gitay, 1972; Holt 62 North, 1970*a*; Iriki, 1941). Additionally, the highly motile parasperm might stimulate the female reproductive tract and play an important role in postmating paternity success (cryptic female choice), as has been suggested for some external stimulatory courtship behaviours (Edvardsson & Arnqvist, 2000). Thus, the motility or volume of parasperm could be a sexually-selected signal that stimulates females to alter the uptake, storage, or use of eusperm.

(*b*) *Capacitation*

Second, it has been suggested that parasperm function to initiate capacitation, the acquisition of motility, by the eupyrene sperm (Osanai, Kasuga & Aigaki, 1987; Osanai & Isono, 1997). The spermatophore of the silkmoth, *Bombyx mori*, acts as the site of maturation of both apyrene and eupyrene sperm (Osanai, Kasuga & Aigaki, 1990). Prior to ejaculation and formation of a spermatophore both sperm types are immotile, although the apyrene sperm have already dissociated. A multi-functional endopeptidase, initiatorin, secreted by the glandula prostica, causes the activation and vigorous motility of apyrene sperm. Initiatorin also kicks off a cascade of reactions, resulting in motile eupyrene sperm; full motility of eupyrene sperm is not attained until after migration to the spermatheca (Osanai, Kasuga & Aigaki, 1989*a*). Based on direct observation, apyrene sperm function to promote the dissociation of eupyrene sperm bundles first, by mechanically breaking the bundles apart and second, by mixing the highly viscous contents of the spermatophore. Without the mechanical action of the apyrene sperm, capacitation would otherwise be retarded (Osanai et al., 1989*a*). Biochemical similarities in the capacitation process have been observed across several orders of insects including those with only one sperm

morph (Osanai & Baccetti, 1993; Osanai & Chen, 1993; Shepard, 1974).

(4) Competition

Insects display a wide array of tactics to ensure or increase paternity (Birkhead & Møller, 1998; Simmons & Siva-Jothy, 1998). Not unlike secondary sexual traits, selection can also act directly on sperm morphology because any sperm trait that enhances competitive ability should be favored in males that possess them (Parker, 1970). Consistent with this idea, insect sperm are remarkably variable with respect to morphology (Jamieson, 1987; Sivinski, 1980). Since Sivinski (1980), many authors have suggested that parasperm might be specialized to provide either defensive or offensive advantage in competition between ejaculates in much the same way as any other morphological feature of an organism might be adapted to provide a competitive advantage against a rival. Four alternative hypotheses have been proposed under the assumption that sperm are in competition.

(*a*) *Elimination*

Silberglied *et al*. (1984) hypothesized that the 'eunuch' sperm (apyrene sperm) of Lepidotera represent specialization of a portion of the ejaculate that serves a competitive function whereby the apyrene sperm would not be involved in fertilization *per se* but would serve to enhance the probability of fertilization by the eupyrene sperm. He specifically enunciated two hypotheses, the first of which he termed the 'elimination' hypothesis. According to the elimination hypothesis, parasperm would be 'preadapted for the seek and destroy (or displace) mission' of removing previously deposited sperm from competition either by flushing a rival males' sperm from the females' sperm storage organs or by incapacitating the sperm through direct interaction. Either way, rival sperm would effectively be removed from competition thus increasing the male's chance at insemination. Most researchers have focused mainly on the flushing aspects of this hypothesis and not paid significant attention to interactions between rival sperm that might lead to incapacitation.

One other potential and as yet uninvestigated mechanism by which parasperm could trigger the elimination or flushing of rival sperm is through interaction with the female. Parasperm could act as a signal, possibly of a healthy eupyrene sperm complement or as an indicator of superior genetic quality, that females could evaluate before retaining or evacuating previously deposited sperm. Some forms of parasperm, such as the apyrene sperm of Lepidoptera, are sufficiently abundant to represent a potential handicap, which would ensure signal reliability. The motility of apyrene sperm might function in a manner similar to tarsal rubbing in beetles (Edvardsson & Arnqvist, 2000), leg tapping in flies (Otronen, 1997), or genital stimulation in odonates (Cordoba-Aguilar, 1999).

(*b*) *Cheap filler*

The second hypothesis proposed by Silberglied *et al*. (1984) was the ''prevention'' hypothesis. According to this hypothesis, parasperm may function as an energetically inexpensive means of filling the spermatheca, cause a delay in female remating, and thereby decrease the risk of sperm competition. Many female Lepidoptera copulate more than once, but after mating females are often not receptive to subsequent mating for a period of days. In Lepidoptera, females have evolved several mechanisms to detect and gauge the size of the male spermatophore, which is deposited in the bursa copulatrix. Females can detect the presence and size of the spermatophore by means of stretch receptors in the wall of the bursa; experimental and correlational studies have shown that larger spermatophores increase the female refractory period (Sugawara, 1979). Further, some evidence points to the refractory period being dependent on the amount of motile sperm transferred to the spermatheca (Benz, 1969; Taylor, 1967; Thibout, 1975, 1979). Silberglied *et al*. (1984) suggested that the smaller, structurally more simple, apyrene sperm are less costly to produce than eupyrene sperm. As apyrene sperm are often produced in large numbers and are highly motile, they might effectively trigger, and circumvent, the female's sperm detection mechanisms. Because of the descriptive nature of the term, most subsequent researchers have referred to this hypothesis as the ''cheap filler'' hypothesis. Interestingly, the basic premise of this hypothesis, that parasperm are less expensive to produce, has never been tested.

(*c*) *Blocking*

Finally, parasperm might function to block access to important areas of the female reproductive tract and exclude successive male's sperm (Baker & Bellis, 1988, 1989). Direct competition with future ejaculates would then be circumvented. Although orig-

inally proposed for mammals, this terminology and hypothesis has later been adopted in insects as well. Woodward (1940) observed in the gastropod, *Giobiosis laqueta* (Say), the agglutination or clumping of apyrene sperm. He suggested that parasperm act as a plug keeping the ejaculate and eupyrene sperm in the female, but the plug could also exclude subsequent sperm.

There is some overlap between the 'elimination', 'cheap filler' and 'blocking' hypotheses in their proposed functions. The end result predicted by all three hypotheses would be similar, i.e. reduced contact and competition between rival fertilization-competent sperm. However, predictions concerning female behaviour (e.g. remating) or male behaviour (e.g. plug removal) might differ substantially. For example, one would predict a positive relationship between the proportion of parasperm and remating latency under the 'cheap filler' hypothesis, but little or no relationship under either the 'elimination' or 'blocking' hypotheses. Patterns of sperm precedence might then be used to distinguish between the 'elimination' and 'blocking' hypotheses; one would predict higher first-male precedence according to the 'blocking' hypothesis but higher second-male precedence according to the 'elimination' hypothesis.

(*d*) *Alternative fertilization strategy*

Finally, for cases when both sperm morphs are nucleated with an appropriate haploid genome (e.g. some *Drosophila* spp. and diopsid flies), it has been suggested that both sperm morphs are capable of fertilizing the egg. The different size classes might represent adaptations for fertilization at different stages of the female's reproductive life or under different mating conditions (Joly, Cariou & Lachaise, 1991; Sivinski, 1980). In a variety of *Drosophila* species both sperm morphs are transferred but they are not distributed randomly within the female reproductive tract. Short sperm are the first to arrive at the spermathecae but long sperm persist longer in the female's reproductive ducts (e.g. Bressac, 1994; Snook, Markow & Karr, 1994). Thus, it has been suggested that the larger gamete is specialized for delayed fertilization (Bressac *et al*., 1991*b*; Joly *et al*., 1991). Because the larger and presumably hardier sperm morph would be better equipped to survive and resist displacement inside the female, it might be also be able to block or eliminate rival sperm (Sivinski, 1980). Short sperm, on the other hand, might be specialized for immediate fertilization (Bressac *et al*., 1991*b*; Joly *et al*., 1991). Thus, each class of sperm might experience differential success with regard to fertilization depending on mating order or mating status of the female. Long sperm would be predicted to be at a selective advantage when females mate multiply (Joly *et al*., 1991), while short sperm would be favoured when females are monogamous (Joly *et al*., 1991; Sivinski, 1980). Joly *et al*. (1991) hypothesized that stable sperm polymorphisms would be found only in facultatively polygamous social systems.

III. DISTRIBUTION AND DEVELOPMENT OF HETEROMORPHIC SPERM

Despite being discovered over a century ago, relatively few data that speak to the functional significance of polymorphic sperm are available. Because the role of parasperm may differ between groups, a reasonable assumption given their different morphologies and apparent independent evolutionary origins, we will review available data separately for each group of insects. In each section, we briefly outline what is known about spermatogenesis and then discuss experiments that shed light on function.

(1) Lepidoptera

(*a*) *Distribution*

The most extensively studied case of sperm dimorphism in insects occurs in the Lepidoptera (Fig. 1, for reviews see Friedlander, 1997; Silberglied *et al*., 1984). Compared to eupyrene sperm, apyrene sperm are also shorter, thinner, and have less mitochondrial content (Friedlander, 1997) with few reported exceptions (M. Gage, personal communication; Etman & Hooper, 1979*a*). Substantial variation in lengths of apyrene (short) and eupyrene (long) sperm were found across a large sample of both butterflies [$N = 70$, apyrene length 216–756 μm, eupyrene length 345–1545 μm, (Gage, 1994)] and moths [$N = 149$, apyrene length 106–883 μm, eupyrene length 110–12675 μm, (Morrow & Gage, 2000)] representing species from a total of five and 17 families, respectively. Both eupyrene and apyrene spermatozoa reach the spermatheca, the site of sperm storage, of fertilized females, but only eupyrene spermatozoa actually fertilize the eggs (Friedlander & Gitay, 1972). Although not involved in fertilization, the apyrene spermatozoa are often produced in large numbers, reaching in excess of

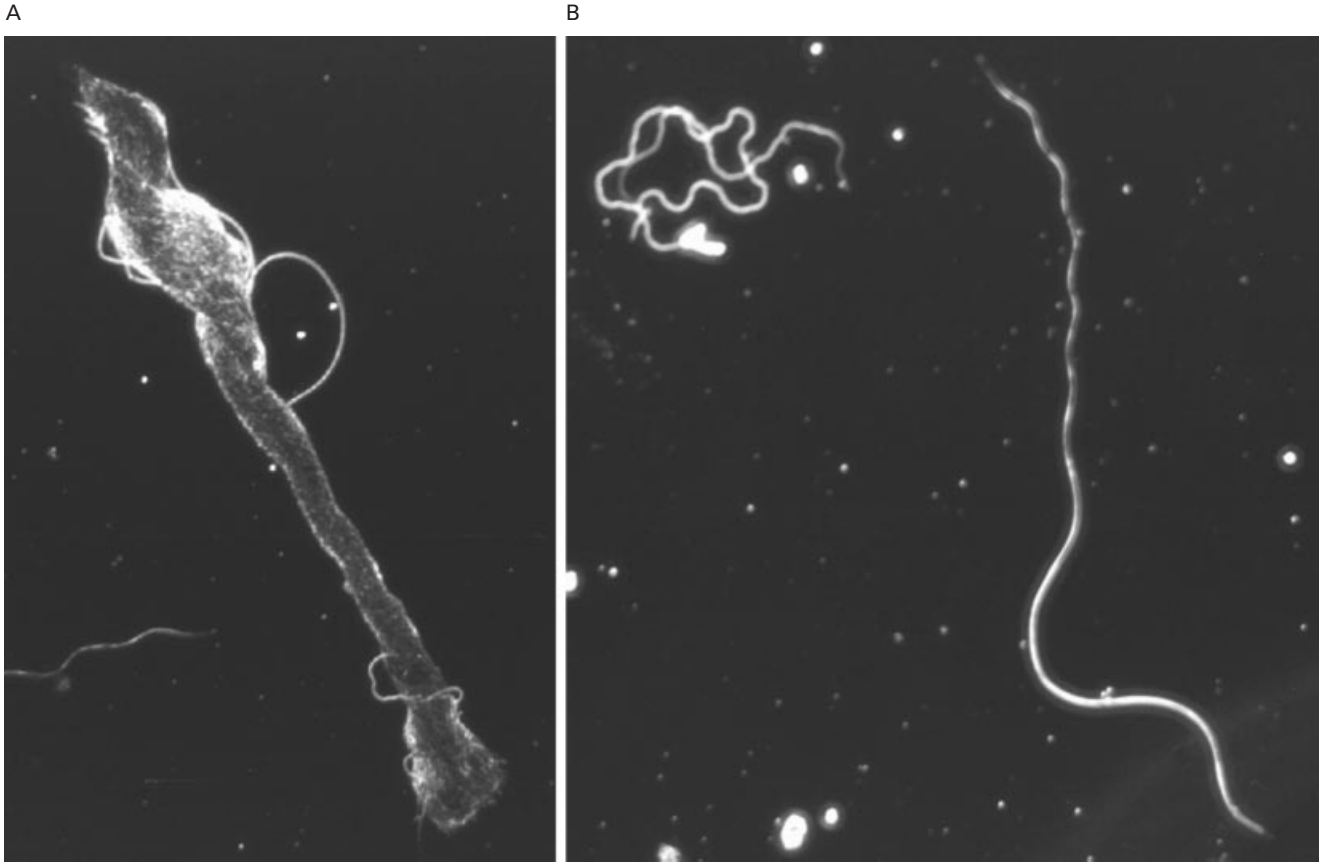

Fig. 1. (A) Eupyrene sperm bundle from the small white butterfly, *Pieris rapae*. Eupyrene sperm are transferred in undissociated, immotile bundles of 256 spermatozoa to the female. Eupyrene sperm bundles continue maturation and dissociate in the inner matrix of the spermatophore, which forms in the bursa copulatrix of females (Osanai & Isono, 1997). Eupyrene sperm do not attain full maturation and capacitation until after emigration from the spermatophore into the spermatheca. (B) Individualized eupyrene sperm and apyrene sperm. Eupyrene sperm are longer (average length = 762 μm) and contain a nucleus while apyrene sperm are shorter (average length = 474 μm) and lack a nucleus. Photographs provided by N. Wedell.

90 % in some species (Table 2; Cook & Wedell, 1996) even when spermatophore volumes are halved due to nutritional stress (Gage & Cook, 1994). As with sperm length, however, the proportion of short sperm produced appears variable between species with some producing less than 10 % (Table 2; J. G. Shepard, unpublished data from dissections of male reproductive tracts).

Dichotomous spermatogenesis appears to be universal throughout the higher Lepidoptera (Friedlander, 1983; Gage, 1994; Hamon & Chauvin, 1992; Morrow & Gage, 2000) and has also been reported in lower Lepidoptera, such as the family Agathaphagidae (Kristensen, 1984). Interestingly, both the presence and absence of apyrene sperm have been reported in the family Micropterigidae, which is widely regarded as the sister-group to all remaining Lepidoptera (Neilsen, 1989; Wiegmann *et al*., 2000). Friedlander (1983) reported the presence of apyrene sperm in *Epimartyria pardella* while only one sperm type was reported in two species from the nominate genus, *Micropterix*, of the family Micropterigidae (Hamon & Chauvin, 1992; Sonnenschein & Hauser, 1990). *Micropterix* is the only genus within the Lepidoptera that appears to lack apyrene sperm. Therefore, unless Micropterigidae is paraphyletic, these results suggest that dichotomous sperm production has evolved at least twice in Lepidoptera. Monophyly of Micropterigidae, including species of the genera *Epimartyria* and *Micropteryx* is strongly supported (100 % bootstrap support) in a recent molecular phylogenetic analysis of the lower Lepidoptera (Wiegmann *et al*., 2000). We were unable to find any studies on the sperm morphology of species in either Heterobathmidae or Eriocranidae. Production of apyrene sperm is absent

Table 2. *Sperm counts, the proportion of parasperm, and lengths in sperm-polymorphic Diptera and Lepidoptera.*

Family	Species	Total	Per cent parasperm	Length (mm) long	Length (mm) short	Reference
Diptera						
Diopsidae	*Cyrtodiopsis dalmanni*	132900	0.09	0.176	0.089	Presgraves (1997)
	C. quingueguttata	96600	0.09	0.183	0.087	Presgraves (1997)
	C. whitei Thailand	71000	0.09	0.192	0.057	Presgraves (1997)
	C. whitei Malaysia	54500	0.19	0.19	0.053	Presgraves (1997)
	Diopsis apicalis	29800	0.15	0.419	0.119	Presgraves (1997)
	D. fumipennis	38400	0.06	0.465	0.119	Presgraves (1997)
	Sphyracephala becarri	25900	0.13	0.352	0.112	Presgraves (1997)
	S. brevicornis	57900	0.16	0.497	0.124	Presgraves (1997)
	Teleopsis quadriguttata	32900	0.24	0.227	0.096	Presgraves (1997)
Drosophilidae	*Drosophila affinis*		0.70	0.424	0.112	Joly & Lachaise (1994)
				0.51	0.13	Snook (1995)
	D. Algonquin			0.52	0.12	Sanger & Miller (1973)
				0.5	0.13	Snook (1995)
				0.894	0.15	Snook (1995)
	D. ambiqua USA			0.313	0.102	Snook (1995)
	D. ambiqua Europe			0.31	0.086	Snook (1995)
	D. athabasca			1.527	0.118	Snook (1995)
	D. azteca			1.875	0.299	Bircher & Hauschteck-Jungen (1997)
			0.88	0.925	0.143	Joly & Lachaise (1994)
				1.433	0.174	Snook (1995)
	D. bifasciata		0.31	0.228	0.083	Joly & Lachaise (1994)
	D. guanache		0.50	0.273	0.131	Joly & Lachaise (1994)
	D. helvetica		0.45	0.223	0.1	Joly & Lachaise (1994)
	D. kitumensis		0.69	0.248	0.087	Joly & Lachaise (1994)
	D. maderiensis		0.66	0.218	0.137	Joly & Lachaise (1994)
	D. microlabis		0.66	0.196	0.068	Joly & Lachaise (1994)
	D. miranda			0.309	0.087	Snook (1995)
	D. obscura		0.34	0.139	0.076	Joly & Lachaise (1994)
				0.23	0.096	Snook (1995)
	D. persimilis		0.40	0.244	0.067	Joly & Lachaise (1994)
				0.325	0.077	Snook (1995)
	D. pseudoobscura		0.41	0.263	0.056	Joly & Lachaise (1994)
		25000*	0.58	0.363	0.092	Snook *et al.* (1994)

	D. subobscura	5000**	0.53	0.199	0.085	Joly & Lachaise (1994)
	D. subobscura USA		0.66	0.448	0.256	Snook (1995)
	D. subobscura Europe		0.66	0.327	0.197	Snook (1995)
	D. tessieri Ivory Coast***			1.60	1.0	Joly *et al.* (1991)
	D. tristis		0.67	0.235	0.112	Joly & Lachaise (1994)
Lepidoptera						
Pieridae	*Pieris rapae*	135000	0.90	0.762	0.474	Cook & Wedell (1996)
	P. napi	57000	0.88			N. Wedell unpublished
Hepialidae	*Hepialus behrensi*	124600	0.11			J. G. Shepherd unpublished
Incurvariidae	*Paraclemensia acerifoliella*	19050	0.21			J. G. Shepherd unpublished
	Tegeticula yuccasella	25420	0.85			J. G. Shepherd unpublished
Psychidae	*Fumaria casta*	17350	0.03			J. G. Shepherd unpublished
Lyonetiidae	*Bucculatrix ainsliella*	9000	0.86			J. G. Shepherd unpublished
Argyresthiidae	*Argyresthia thuiella*	24500	0.56			J. G. Shepherd unpublished
Papilionidae	*Papilio polyxenes*	437000	0.91			J. G. Shepherd unpublished
Geometridae	*Alsophila pometaria*	20020	0.14			J. G. Shepherd unpublished
Sphingidae	*Manduca Sexta*	2401000	0.95			J. G. Shepherd unpublished
	Pachysphinx modesta	10329000	0.99			J. G. Shepherd unpublished
Arctiidae	*Spilosoma virginica*	525500	0.92			J. G. Shepherd unpublished
Gelechidae	*Pectinphora possypiella*	106000	0.55			LaChance, Richard & Proshold (1975)
Lymantriidae	*Lymantria dispar*	511300	0.87			J. G. Shepherd unpublished
Noctuidae	*Heliotis virescens*	180800	0.74			Proshold *et al.* (1975)
	Leucania pseudargyia	502000	0.80			J. G. Shephard unpublished
	Pseudaletia separata	293900	0.93	1.4	0.5	He *et al.* (1995)
	Spodoptera litura	1052640	0.47			Etman & Hooper (1979*a*)
	Trichoplusia ni	177778	0.46			Holt & North (1970*b*)
Pyralidae	*Plodia interpunctella*	138200	0.93	0.783	0.37	Gage & Cook (1994)
	Ephestia kuehniella	126000	0.89			Marec *et al.* (1995)

* Number of sperm in ejaculate reported in Markow (1996).

** Number of sperm in spermatophore from Bircher *et al.* (1995).

*** Length based on sperm cyst rather than fully elongated sperm.

Sperm counts reported by J. G. Shepard are from dissections of male seminal vesicles and therefore may not be strictly comparable to spermatophore counts from the female bursa copulatrix.

from the closely related sister order Trichoptera, the caddisflies (Friedlander, 1983).

(*b*) *Development*

As control of spermatogenesis and the developmental phases of apyrene and eupyrene sperm in Lepidoptera have been amply reviewed (Friedlander, 1997), only a brief overview will be provided here. The spermatocytes are bipotent giving rise to both sperm types. Spermatogenesis follows a regular pattern (with some variation across species) that is correlated with ontogeny. Eupyrene spermatogenesis begins during the larval phase (as early as the second of five larval instar stages in some species but at later larval stages in other species) and ends at pupation, while apyrene spermatogenesis begins close to pupation (Friedlander, 1997; Holt & North, 1970*a*; Leviatan & Friedlander, 1979). The transition from eupyrene to apyrene sperm development is apparently caused by a haemolymph factor (apyrene-spermatogenesis-inducing factor, ASIF) that becomes active around pupation (Jans, Benz & Friedlander, 1984).

The process of eupyrene spermatogenesis does not deviate from the pattern commonly seen among insects that normally produce only one morphological class of sperm (Baccetti & Afzelius, 1976). How spermatogonia transform into spermatocytes in insects is not known, but this process occurs after a fixed number (n) of spermatogonial divisions followed by two meiotic divisions leading to a predetermined number of sperm (2^{n+2}) per bundle. Species exhibit a characteristic number of divisions with some variation between species. Typically, in Lepidoptera a total of 256 individual spermatids develop within each sperm bundle. Apyrene spermatogenesis is more irregular. It is characterized by a shorter meiotic prophase and by asymmetric and asynchronous distribution of chromosomes during the metaphase and telophase of meiosis (Friedlander & Wahrman, 1970; Wolf, 1994). The resulting apyrene spermatids contain an unbalanced number of chromosomes, which are eventually discarded prior to the end of spermatogenesis (Friedlander & Meisel, 1977). Under normal circumstances spermatogenesis proceeds reliably for eupyrene sperm, but the process can be disrupted by genetic (e.g. hybrid crosses; Richard, LaChance & Proshold, 1975) and environmental factors (e.g. temperature; Lum, 1977). The end stage of ontogeny for apyrene sperm, on the other hand, is not as easily disrupted (Friedlander, 1997).

(*c*) *Functional evidence*

(i) *Provisioning*

That females can receive nourishment from accessory substances in male ejaculates that increase fecundity, egg quality, or longevity has been well established (e.g. Boggs, 1981*a*, 1981*b*; Boggs & Gilbert, 1979). Whether apyrene sperm function in this way is undetermined but appears doubtful for a variety of reasons. First, ample non-sperm nutrient donations are provided in the spermatophore of paternally investing male insects (e.g. Wedell & Cook, 1999*a*). Second, Silberglied *et al.* (1984) dismissed this hypothesis as unlikely because apyrene sperm do not appear to carry any nutrient reserves. Finally, if apyrene sperm contribute nutrients to females, we might expect their production to be sensitive to male condition. Experimentally induced nutritional stress in the moth *Plodia interpunctella* reduced overall spermatophore size but did not affect the relative contribution of apyrene sperm to the female (Gage & Cook, 1994). Nutrient donation from supernumerary sperm to the egg has yet to be investigated.

(ii) *Transportation*

While apyrene sperm generally emigrate from the testes before eupyrene sperm and could, therefore, be in a position to perforate the testicular basement membrane and facilitate emigration of eupryene sperm, as proposed by Katsuno (1977), the subsequent activation and migration of apyrene sperm in the female reproductive tract is left unexplained by this hypothesis. It also appears unlikely that apyrene sperm play a role in transporting eupyrene sperm. Observations of sperm in the reproductive tract do not show close association between sperm types during migration, as would be predicted by the second component of the transportation hypothesis (e.g. Etman & Hooper, 1979*a*; Friedlander & Gitay, 1972; Holt & North, 1970*a*).

(iii) *Capacitation*

The capacitation hypothesis has received the least attention and has yet to be tested beyond the observations made by Osanai's lab (e.g. Osanai *et al.*, 1987, 1990; Osanai & Isono, 1997). Clearly, capacitation of apyrene and eupyrene sperm are functionally associated (see Section II. 3*b*). This result may not be surprising, considering that the processes occur simultaneously within the same ejaculate for both sperm morphs. The question that needs to be addressed in the future is whether the

functional association is the cause or consequence of the evolution of multiple sperm types. The fact that the two sperm types vary independently when males encounter different levels of sperm competition or mates of different age suggests the latter (Cook & Gage, 1995). Regardless, apyrene sperm participating in sperm competition would not necessarily preclude a simultaneous function facilitating capacitation of eupyrene sperm, and *vice versa*.

(iv) *Elimination*

Based on the idea that the last male to mate achieves a higher fertilization rate in butterflies, Silberglied *et al*. (1984) suggested that 'apyrene' sperm play a role in displacing or flushing stored sperm from the spermatheca. However, data from sperm precedence experiments show that second-male precedence (as would be predicted under the elimination hypothesis) is not the rule in Lepidoptera; first-male precedence is not uncommon and sperm mixing is typical. Data compiled by Simmons and Siva-Jothy (1998, see their Table 10.1 p. 352) shows that across Lepidoptera, the mean ($\pm$S.E.M.) proportion of offspring sired by the second male (P_2) is 0.65 ± 0.05. Furthermore, within a given species, P_2 was bimodally distributed with peak values at 0 and 1 for 11 out of 17 species reported. Silberglied *et al*.'s (1984) observation clearly is not a general pattern, eroding support for the elimination/flushing hypothesis.

Even so, apyrene sperm may be involved in sperm displacement in those species in which a high P_2 is observed. For example, experiments by Pair, Laster & Martin, (1977) using hybrids and backcrosses of *Heliothus subflexa* and *H. viriscens* provide evidence for sperm displacement. Backcrossed *H. viriscens* males are sterile, producing normal apyrene sperm but morphologically abnormal eupyrene sperm (Richard *et al*., 1975) that do not reach the female spermatheca (Proshold & LaChance, 1974; Proshold, LaChance & Richard, 1975). Patterns of sperm precedence and female fecundity were then investigated using alternate sterile and normal male matings (Pair *et al*., 1977). Females mated to sterile males after an initial mating to a fertile male showed a 78% reduction in fecundity compared to females mated first to sterile males and later to fertile males. Dissections of the sperm storage organs and sperm counts of doubly mated females showed that nearly 90% of stored eupyrene sperm were displaced following insemination by infertile males. Based on these results, Pair *et al*. (1977) inferred that apyrene sperm flush stored sperm from the spermatheca.

Subsequent studies cast doubt on the general validity of the flushing hypothesis. Later experiments (Etman & Hooper, 1979*b*) investigating the mechanisms of last-male sperm precedence do find support for displacement by an unidentified physiological mechanism. In *Spodoptera litura*, a second mating resulted in the near complete expulsion of stored sperm within 30 min after the completion of copulation. However, following the second mating, stored sperm were flushed from the spermatheca for 15–30 min prior to the arrival of the first sperm from the second copulation. According to Etman and Hooper (1979*b*) sperm flushing commenced prior to the completion of the second copulation leading them to postulate that the act of mating initiates a physiological response responsible for the expulsion of stored sperm. Thus, sperm flushing could not act by physical interactions between rival sperm. However, this result does not rule out the possibility that apyrene sperm act as an indirect signal to the female, causing her to dump previously stored sperm (see Section III. 4*a*).

As a final piece of evidence against the elimination hypothesis, Cook and Gage (1995) found that in *Plodia interpunctella* the numbers of sperm in storage had no effect on the number of apyrene sperm in the ejaculate even though the number of eupyrene sperm increased. Males do appear capable of tailoring their ejaculate in response to risk of sperm competition, as evidenced by the change in eupyrene sperm numbers (Gage, 1995) and even ejaculate size and sperm number (Wedell & Cook, 1999*b*) in relation to female mating status. If apyrene sperm were involved in sperm displacement, they would be predicted to increase in number with the probability and number of previously stored sperm. In fact, the opposite is true (see below). Thus, even if sperm flushing/displacement provides a viable explanation in those species that display second-male sperm precedence, evidence for apyrene sperm performing this function is weak.

(v) *Cheap filler*

Silberglied *et al*. (1984) also proposed that apyrene sperm function to delay female remating, and thereby decrease the chance of sperm competition. Insight into the functional significance of different sperm morphs can be gleaned from information on within-species variation in patterns of ejaculate production, storage and utilization. He, Tanaka & Miyata (1995) suggested that the remating latency of female *Pseudoletia separata* is correlated with the

duration of apyrene sperm storage but not with storage of the longer-lived eupryene sperm. Furthermore, Cook and Gage (1995) found that males transfer a higher proportion of apyrene sperm to young virgin females than to previously mated females. These results are consistent with apyrene sperm increasing the refractory period by acting as ''cheap filler''.

Female reproductive tract morphometry would be expected to show a stronger correlation with apyrene than eupyrene sperm if apyrene sperm act as 'cheap filler' (Gage, 1994). Across 74 species of butterflies, Gage (1994) found that apyrene sperm length was closely associated with female body size, which he suggested might be a proxy for reproductive tract morphometry. In a subsequent comparison of 135 species of moths, Morrow and Gage (2000) found an association not between apyrene sperm length and reproductive tract morphometry but between eupyrene sperm length and spermathecal duct length. Neither apyrene nor eupyrene sperm length correlated with the volume of the female storage organs. Finally, apyrene sperm length was not correlated with the risk of sperm competition (female remating frequency), which is counter to predictions not only of the 'cheap filler' hypothesis but also any of the sperm competition hypotheses.

However, the relative proportions of sperm produced, rather than the length of individual sperm, may more accurately reflect how well a male is able to influence a female's intercopulatory interval. A very large portion of each spermatophore, as much as 99%, is dedicated to apyrene sperm (Table 2). Unfortunately, few studies report the proportion of each sperm morph in the ejaculate. The fact that males deliver larger spermatophores to larger females (Gage, 1998) and that relative testis and accessory gland sizes, the two primary contributors to the spermatophore, are positively associated with spermatheca and bursa copulatrix volume across species of moths (Morrow & Gage, 2000) lends support to this idea. However, residual apyrene sperm number (controlled for eupyrene sperm number) did not show an increase with either female size or spermatophore size (Gage, 1998), in contrast to predictions if apyrene sperm numbers function to delay remating.

Although comparative studies do lend equivocal support for the 'cheap filler' hypothesis, results from these studies should be interpreted with caution for several reasons. First, because sperm polymorphism has probably arisen only once in the common ancestor of all higher Lepidoptera, the function of short sperm must be inferred from covariation between sperm length or number with mating strategies or female reproductive tract dimensions not from independent evolutionary events. Second, some of the results may be confounded in the sense that apyrene and eupyrene sperm length are correlated across species. Given such a correlation, any association seen with eupyrene sperm length (i.e. with polyandry) might also be expected with apyrene sperm length.

The best evidence for the 'cheap filler' hypothesis is provided by an experiment by Cook and Wedell (1999) in which they mated females with either virgin or mated males and then allowed females to remate; mated males produced smaller ejaculates that have a higher proportion of eupyrene sperm (Wedell & Cook, 1999*b*). They then investigated female receptivity to a second mating in relation to the number of stored sperm. Remating latency was positively correlated with the number of stored apyrene sperm. The number of stored eupyrene sperm had no effect on remating latency. Cook and Wedell (1999) concluded that males have circumvented a female system designed to detect sperm numbers by filling the sperm storage organs with a 'cheaper' facsimile.

This interesting result has yet to be replicated in other species that display variation in refractory period or in per cent apyrene sperm (see Table 2). Replication using an alternative means of adjusting male ejaculate would be desirable, because the proportion of apyrene sperm was not independent of spermatophore size in the Cook and Wedell (1999) study. In that study, mated males delivered ejaculates that had higher proportions of eupyrene sperm but produced smaller ejaculates because they did not have time to recover their sperm volume. Females paired initially with mated males received smaller spermatophores (3.6 *versus* 6.5 mg), were more likely to remate (12 of 14 *versus* 13 of 22 pairings), and remated sooner (3.4 *versus* 5.5 days) than females paired initially with virgin males. While mating latency was correlated with per cent apyrene sperm in storage, it was also correlated with the size of the spermatophore delivered by the male (Cook & Wedell, 1999). confounding their results.

(2) Diptera: Drosophilidae

(*a*) *Distribution*

Among the Diptera, all 17 species and two subspecies of the *Drosophila obscura* subgroup (Barrio & Ayala,

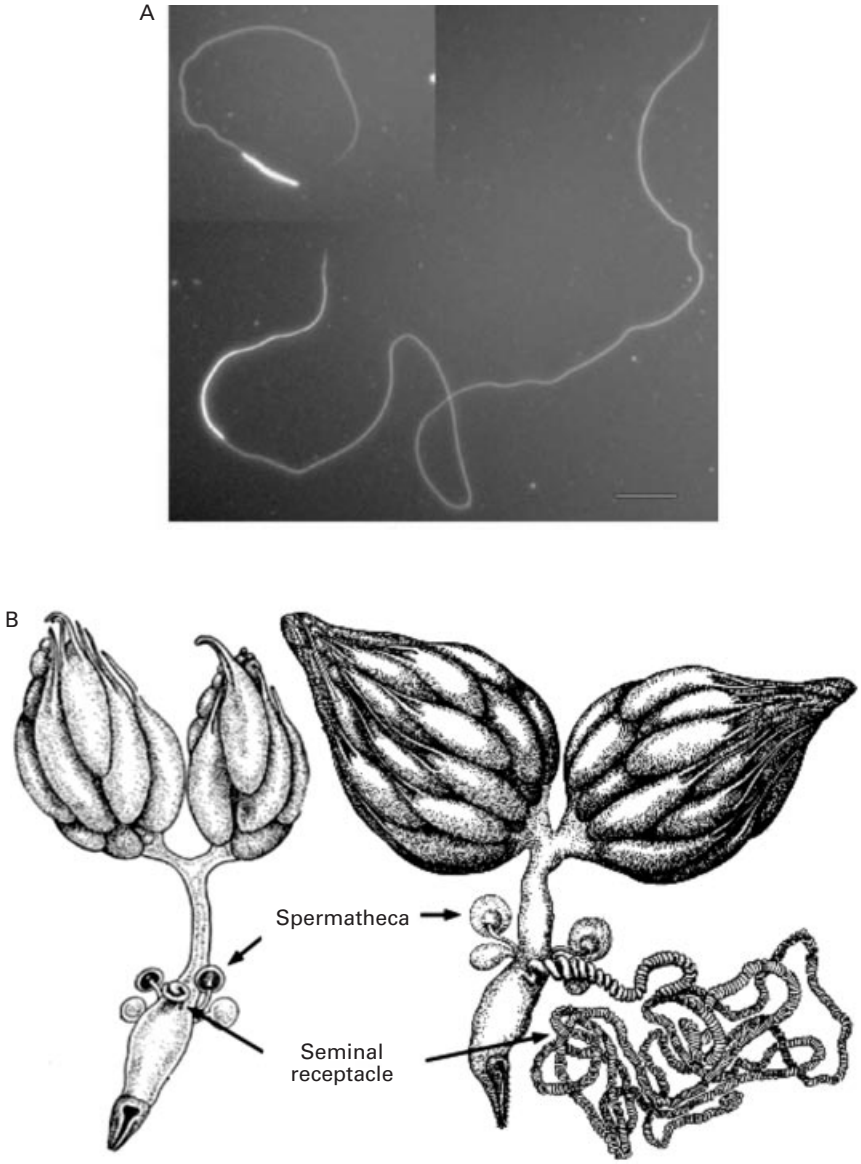

Fig. 2. (A) Individual long and short sperm, stained with Hoechst, from the seminal vesicles of a reproductively mature *Drosophila affinis*. Both the long and the short morph are nucleated: visualized as the brightly fluorescing ends of the sperm. Epiflourescence microscopy clearly reveals that the difference in total length between the long and short morph is a function of differences in both head and tail length. Average length of *D. affinis* long sperm is 0.467 mm; the average length of short sperm is 0.121 mm (Table 2). Scale bar 20 μm. (B) Drawings of the female reproductive tract of *D. pseudoobscura* (left), which produces 0.36 mm long sperm, and *D. bifurca*, which produces a single sperm morph of 58.29 mm (right). The length of the seminal receptacles are 0.41 mm and 81.67 mm, respectively, and show correlated evolution with the length of the fertilizing sperm (Pitnick & Miller, 2000). Photographs provided by R. R. Snook. Drawings by J. T. Patterson, adapted from Pitnick and Miller (2000).

1997) that have been examined to date (Beatty & Sidhu, 1970; Joly *et al*., 1989; Joly & Lachaise, 1994; Policansky, 1970; Sanger & Miller, 1973; Snook & Karr, 1998; Snook *et al*., 1994) produce two discrete lengths of nucleated, motile sperm, a form of sperm polymorphism termed polymegaly (Fig. 2); The *Drosophila obscura* subgroup is a monophyletic cluster comprised of the approximately 55 species within Drosophilidae (Barrio & Ayala, 1997). The two morphs differ in both head and tail length (Beatty & Burgoyne, 1971; Bircher & Hauschteck-Jungen, 1997; Joly & Lachaise, 1994). While several authors have reported three or more discrete length morphs (e.g. Beatty & Sidhu, 1970; Bircher & Hauschteck-Jungen, 1997; Joly & Lachaise, 1994; Policansky, 1970; Takamori & Kurokawa, 1986), the distri-

butions of what might be considered the short and medium morphs show a high degree of overlap while the distribution of the long morph is discontinuous from the remainder. Consequently, many authors generally recognize two sperm length morphs (e.g. Pasini *et al*., 1996; Snook *et al*., 1994). *Drosophila* in the *obscura* group display more variation in the length of the long sperm morph (139–1875 μm) than the short sperm morph (56–299 μm; Table 2). In general, long morph sperm from the *obscura* group are short compared to sperm-monomorphic *Drosophila* species (Bircher & Hauschteck-Jungen, 1997; Bressac *et al*., 1991*a*; Joly & Lachaise, 1994, Markow, 1996; Pitnick, Markow & Spicer, 1999). Species in the *Drosophila obscura* subgroup also vary considerably in the proportion of each sperm type produced in the testes (31–88 % short sperm; Table 2) and, in the case of *D. subobscura*, the proportion of sperm bundles produced in the mature testes correlates with the proportion of each sperm type delivered to females (Bircher *et al*., 1995).

Polymegaly has only been reported for one other species of *Drosophila* outside the *obscura* group, *Drosophila tessieri* (Table 2; Joly *et al*., 1991). However, unlike in the *obscura* subgroup where all species display a discrete polymorphism for sperm length, distribution of sperm lengths in *D. tessieri* is bimodal in two populations but unimodal in one population. Sperm length in the sperm-monomorphic population is comparable to the long morph of the other two populations.

(*b*) *Development*

Spermatogenesis in *Drosophila obscura* is characteristic of most Diptera, with large numbers of individual spermatids developing within a pair of cyst cells to form a spermatocyst or ''sperm bundle''. Five synchronous mitotic divisions are followed by two meiotic divisions (Fuller, 1993; Takamori & Kurokawa, 1986). Each resulting sperm bundle contains 128 spermatids. Both morphological classes of sperm result from similar regular development of spermatogonia and both are nucleated. Thus, polymegaly differs from the sperm dimorphism exhibited in Lepidoptera in that both sperm types carry what appears to be a full complement of chromosomes. Sperm length varies between, but not within, sperm bundles (Beatty & Burgoyne, 1971), so the long and short sperm develop separately. The factors determining the developmental fate of each type are unknown.

During spermatogenesis, both sperm types undergo a nuclear transition in which the somatic histones are replaced by sperm-specific arginine-rich nucleoproteins (Hauschteck-Jungen and Rutz, 1983). Although the exact function is unknown, this transformation appears to be essential for normal sperm function. Sperm-monomorphic *Drosophila* that lack nucleoproteins demonstrate sperm dysfunction (Hauschteck-Jungen & Rutz, 1983). Furthermore, in Lepidoptera the apyrene (non-fertilizing) sperm do not undergo the histone transition while the eupyrene sperm do (Friedlander & Hauschteck-Jungen, 1982). Based on these results, Hauschteck-Jungen and Rutz (1983) suggested that both sperm types might be fertilization competent.

Other than the obvious length differences, the ultrastructure of the two morphs is quite similar. Takamori and Kurokawa (1986) noted some slight irregularities in the head region of the short sperm of *Drosophila bifasciata*. Similarly, Pasini *et al*. (1996) noted only minor differences between the two sperm types in acrosome size, nucleus morphology, and the relationship between the nucleus and minor mitochondrial derivatives in *Drosophila subobscura*. Based on the similarities of the ultrastructural characters, cytochemical characters and DNA content, these authors also concluded that both sperm morphs are potentially capable of egg penetration and fertilization.

(*c*) *Functional evidence*

Bressac *et al*. (1991*b*) demonstrated differential patterns of sperm motility and activation in the *Drosophila obscura* group. Long sperm underwent an increase in activity in the female storage organ. They proposed that this ''over-activation'' confers on long sperm a greater ability to survive and resist displacement in the female reproductive tract. Taken together with results from crosses of sperm monomorphic and dimorphic populations of *Drosophila tessieri*, Joly *et al*. (1991) proposed that sperm dimorphism is an adaptation for polyandry because each sperm type fares better in competition under different mating circumstances. According to their hypothesis, short sperm are adapted for immediate fertilization. Thus, short sperm would be the first to be utilized by singly mated females or would gain immediate fertilization success in females that are storing sperm from a previous mating. Long sperm would be preferentially used when females begin to use sperm in long-term storage.

Mounting evidence indicates that short sperm are not used for fertilization in the sperm heteromorphic

Drosophila obscura group. Despite a tendency for short sperm to arrive first at the sperm storage organs (Bressac, 1994; Snook *et al*., 1994), several groups have shown that only long sperm persist in the female storage organs and that patterns of sperm usage suggest that only long sperm are used for fertilization in *Drosophila pseudoobscura* and *Drosophila subobscura* (Bressac & Hauschteck-Jungen, 1996; Snook *et al*., 1994). Using sperm-specific antibodies and digital deconvolution microscopic analyses of fertilized eggs, Snook *et al*. (1994) showed that only long sperm participate in fertilization in *D. pseudoobscura*. This same technique has subsequently been repeated on five additional species with the finding that only long sperm participate in fertilization in *D. affinis*, *athabasca*, *miranda*, *persimilis*, and *subobscura* (Snook & Karr, 1998). This result is compelling because it held under a variety of mating and fertilization conditions, including multiple partners and polyspermy. Finally, Snook (Snook, 1998*a*) showed that the infertile hybrid between multiple populations of two subspecies of *D. pseudoobscura* (*D. p. pseudoobscura* females × *D. p. bogotana* males) produce nearly 100% short sperm, all of which are immotile. The reciprocal hybrid males are fertile and produce approximately 40% long sperm (significantly more short sperm than two of four parental strains). Together, these results indicate non-equivalence of the two sperm types allowing us to reject all hypotheses that assume that both sperm morphs fertilize eggs. Short sperm morphs did not participate in immediate fertilization of eggs; short sperm were not utilized by either singly mated females (Snook *et al*., 1994) or by multiply mated females (Snook & Karr, 1998). Snook and Karr (1998) provide two testable hypotheses as to why the short sperm are fertilization incompetent. First, short sperm might have biochemical incompatibilities that do not permit proper interaction with the egg. Second, short sperm may have physical incompatibilities with the egg, e.g. the head of the short sperm may be too wide to enter the micropyle of the egg. Neither has yet been tested.

Given that short sperm do not appear to function as gametes, we need to consider other selective forces that could maintain the dimorphism. As discussed above, several major sets of hypotheses have been proposed for the function of non-fertilizing sperm. None of these has received any empirical support in *Drosophila*. Following the fate of ^{14}C-radiolabeled male seminal fluids, Snook and Markow (1996) found no association between the disappearance of short sperm and male-derived tissues that were incorporated into either female somatic tissues or oocytes. As reported above, subsequent studies confirmed that, even though polyspermy is not uncommon in *Drosophila*, short sperm do not enter the egg (Snook & Karr, 1998), further diminishing the possibility for a nutritive role.

Similarly, there is no support for any of the sperm competition hypotheses. Short sperm do not appear to ''block'' access to the female storage organs, as they do not survive in the female reproductive tract when females are again receptive (Snook, 1998*b*; Snook *et al*., 1994). Sperm-dimorphic *Drosophila* also do not appear to adjust the composition (measured as ratio of long to short sperm) of their ejaculate based on the risk of sperm competition (mated *versus* unmated females or presence of rival males; Snook, 1998*b*) as do some Lepidoptera (e.g. Cook & Gage, 1995). The presence of short sperm also did not significantly affect female remating latency (Snook, 1998*b*) a key prediction for the 'cheap filler' hypothesis (Silberglied *et al*., 1984). Finally, *Drosophila* short sperm do not appear to show correlated evolution with spermathecae size and number (Fig. 2; Pitnick *et al*., 1999). The probability of female remating was correlated with the absence of an egg in the uterus, but not with any component of the male ejaculate (Snook, 1998*a*).

(3) Diptera: Diopsidae

(*a*) *Distribution*

In a survey of 13 species of stalk-eyed flies, family Diopsidae, nine species were found to produce two non-overlapping size classes of nucleated sperm (Fig. 3; Presgraves, Baker & Wilkinson, 1999). Phylogenetic analysis reveals that sperm dimorphism represents the ancestral state in the family, and therefore possibly occurs in sister groups to the family Diopsidae. Average sperm lengths of short and long sperm size classes are 124 and 497 μm, respectively, in *Sphyracephala brevicornis*, a species from the most basal lineage in the family (Baker, Wilkinson & DeSalle, 2001), and 53 and 192 μm in *Cyrtodiopsis whitei* (Table 2). The proportion of mature sperm cysts in the short size class varies from 24% in *Teleopsis quadriguttata* to 6% in *Diopsis fumipennis* (Table 2). An alternative sperm production strategy is found in species from the African genus *Diasemopsis*. In every one of the four species examined from this genus, males produce a single size class of sperm, which is longer than the long sperm morph from any of the sperm dimorphic

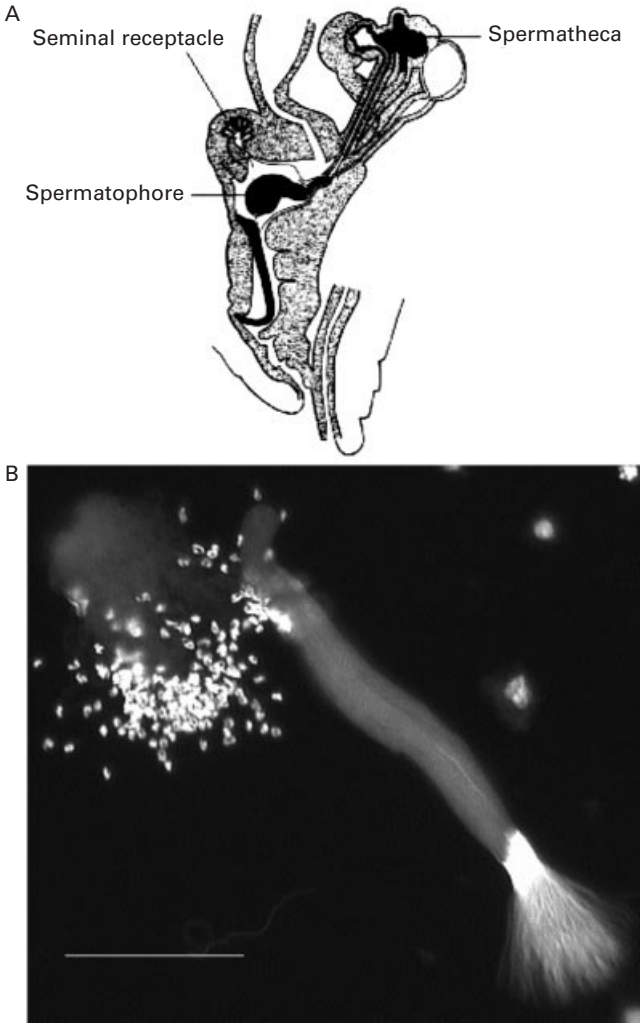

Fig. 3. (A) A schematic illustration of the female reproductive tract of *Cyrtodiopsis whitei*. All sperm-dimorphic stalk-eyed flies, such as *C. whitei*, have three spermathecae while sperm-monomophic stalk-eyed flies have only two, relatively degenerate spermathecae (Presgraves *et al*., 1999). Female reproductive tract adapted from Kotrba (1995). (B) Long and short sperm bundles from the testes of a male *C. whitei*. There is little variation within a sperm bundle; most of the variation in sperm length is between bundles. Both sperm morphs are nucleated. Sperm bundles were visualized with Hoechst stain using epiflourescence. Scale bar 50 μm. Photograph provided by J. Edwards.

species, Average sperm lengths vary from 920 μm in *Diasemopsis munroi* to 2988 μm in *D. aethiopica*.

(*b*) *Development*

Spermatogenesis in sperm dimorphic-diopsid species, such as *Cyrtodiopsis whitei*, is characteristic of other Diptera (Lindsley & Tokuyasu, 1980; Roosen-Runge, 1977), with 128 spermatids per sperm bundle. All sperm within a bundle either form long or short morph sperm (see Fig. 3). Spermatogenesis occurs after eclosion resulting in delayed male reproductive maturity. For example, *Cyrtodiopsis dalmanni* and *C. whitei* males do not produce individualized sperm until three weeks of age. The total number of mature sperm bundles in the testes of male *C. whitei* continues to increase until at least eight weeks of age (Wilkinson & Sanchez, 2001). Male *C. dalmanni* allowed access to females for two weeks prior to dissection had more mature sperm bundles at four weeks of age than males housed without females, suggesting that either female presence or mating activity accelerates spermatogenesis (J. Edwards, unpublished data). Although the proportion of sperm bundles in the small morph size class is low in all diopsids (Table 2), it does not vary between testes within a male or between males ranging from four to 21 weeks of age. The proportion of short morph sperm is, however, slightly higher in males housed without females, compared to those housed with females, as expected if short morph sperm develop and accumulate faster than long morph sperm, as has been reported for *Drosophila pseudoobscura* (Snook, 1998*b*; Snook *et al*., 1994) and *D. subobscura* (Bircher *et al*., 1995).

Some sperm-dimorphic diopsids, such as *Cyrtodiopsis dalmanni* and *Cyrtodiopsis whitei*, harbour sex chromosome meiotic drive (Wilkinson, Presgraves & Crymes, 1998*b*). Males that carry a driving X-chromosome produce predominantly female offspring (Presgraves, Severance & Wilkinson, 1997) presumably because Y-bearing sperm are disabled during development. This phenotype closely resembles the Sex Ratio (SR) trait described for many *Drosophila* species (Jaenike, 1996; Lyttle, 1991), including several sperm-dimorphic species in the *obscura* group. Bircher *et al*. (1995) reported that SR *D. subobscura* males produce more sperm, by producing more short morph sperm, than non-SR mates and females that copulate with SR males store more long sperm than females that copulate with non-SR males. In contrast to these results, neither the total number of sperm nor the proportion of short sperm differs between SR and non-SR male *C. whitei* matched for age (Wilkinson & Sanchez, 2001). In addition, related diopsid species that lack sex chromosome meiotic drive, such as *C. quinqueguttata* (Wilkinson *et al*., 1998*b*), exhibit similar proportions of short morph sperm to those which carry it (Table 2). Thus, there appears to be no developmental or selective association between sex chromosome meiotic drive and sperm dimorphism in diopsids.

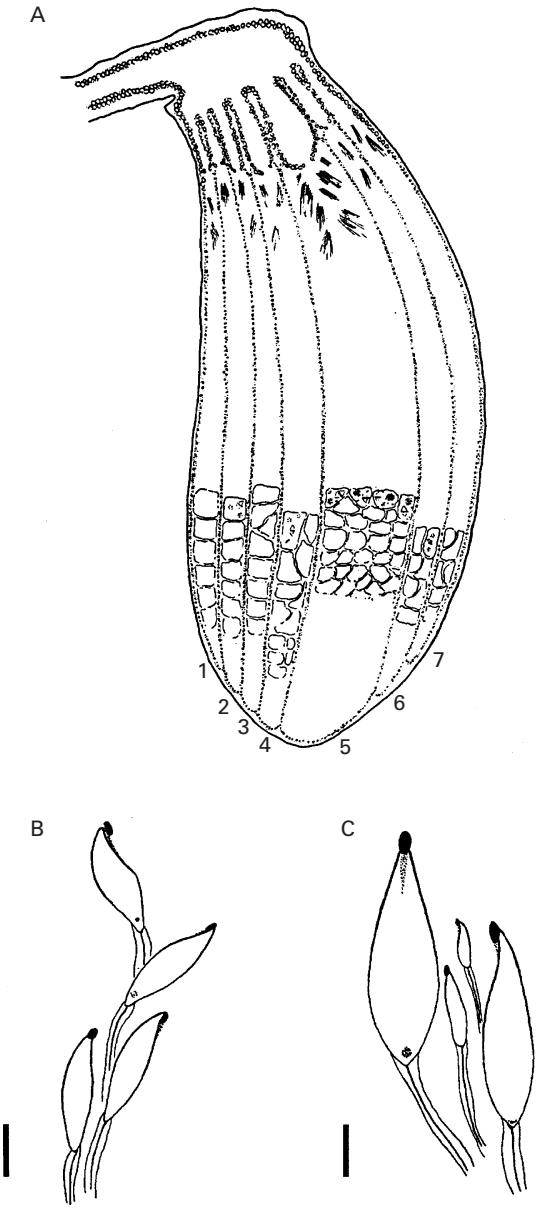

Fig. 4. (A) Drawing of a cross section of the testes of *Loxa flavicollis* illustrating the seven testicular subdivisions (lobes), including relative position and size. In this species, the "harlequin lobe", which produces heteroploid sperm, is the fifth lobe. Testes length is approximately 4 mm. (B, C) Drawings of sperm heads from *L. flavicollis*. Sperm heads from the six non-"harlequin lobes" are of uniform size, while sperm heads from the "harlequin lobe" vary in size. The smallest sperm contain a single sex chromosome; the largest contain up to 200 chromosomes. Scale bar approximately 5 μm. Drawings adapted from Schrader (1945*a*).

(*c*) *Functional evidence*

Direct evidence implicating short sperm in fertilization is not available for any diopsid. As in *Drosophila* species (Karr & Pitnick, 1996), flourescent antibody-labeled sperm have been observed inside an embryo in at least one sperm-monomorphic diopsid, *Diasemopsis aethiopica* (T. Karr, unpublished data). However, attempts to quantify the length of fertilizing sperm in a sperm-dimorphic diopsid have not yet been successful. Although short morph sperm have broader heads than long morph sperm, short morph sperm are not obviously excluded from entering eggs. Scanning electron microscopy examination of egg morphology in 27 diopsid species (Meier & Hilger, 2000) fails to indicate any morphological difference in the size of the micropyle, the opening at the tip of the egg through which sperm must pass for fertilization, between sperm monomorphic and sperm dimorphic species. In all species examined, the diameter of the micropyle is approximately 5 μm, while the diameter of the head of both sperm morphs is typically less than 1 μm.

Short morph sperm seem likely to exhibit selective influence on some aspect of sperm storage since the length of the short sperm exhibits correlated evolution with the size of the spermathecae, the primary sperm storage organs in female sperm-dimorphic diopsids (Fig. 3; Presgraves *et al.*, 1999). Short morph sperm length did not exhibit correlated evolutionary change with either spermathecal duct length or ventral receptacle length – the site of fertilization. The length of long morph sperm, on the other hand, shows correlated evolution with the length of the spermathecal duct and with ventral receptacle length, consistent with a putative role for long morph sperm in fertilization. Additional evidence implicating short morph sperm in sperm storage interactions comes from examination of the female reproductive tracts in sperm monomorphic diopsids. All species of *Diasemopsis* examined to date have two, rather than three, spermathecae, and in many cases, the spermathecae are drastically reduced in size and no longer appear to function as sperm storage devices (Fig. 3; Kotrba, 1995; Presgraves *et al.*, 1999). The ventral receptacle, by contrast, has become hypertrophied in these species, often with several hundred tube-like chambers each capable of containing a single, long coiled spermatid (Kotrba, 1995). Thus, short morph sperm occurs in those species that store sperm in an organ that permits sperm to interact. When sperm are effectively isolated from interaction, short sperm are not present. These comparative data provide compelling evidence for short sperm function related to female sperm storage.

Unfortunately, multiple hypotheses remain, i.e.

short morph sperm could act to fill spermathecae, block competitive sperm movement, provide nutrition or aid movement of long morph sperm. The ''cheap filler'' hypothesis seems doubtful, however, because relatively few short sperm tend to be produced by any diopsid (Table 2), female *Cyrtodiopsis whitei* require four to five matings before their spermathecae are filled (Lorch, Wilkinson & Reillo, 1993), and female *Cyrtodiopsis* of all species are highly promiscuous and exhibit no decline in mating frequency even after mating enough times to fill their sperm storage organs multiple times (Wilkinson, Kahler & Baker, 1998*a*). Unless males mate in rapid succession, in which case the spermatophore of the first male appears to block subsequent sperm transfer (Lorch *et al*., 1993), sperm effectively mixes in the spermathecae (Lorch *et al*., 1993) and diminishes male paternity accordingly in *C. whitei*. Indirect experimental evidence is consistent with the sperm-blocking hypothesis. Doubly-mated female *C. whitei* show a negative association between the body size of the first male mated and the defensive capability of his sperm (P1). Short morph sperm could be involved in this precedence effect because the length of short sperm also covaries negatively with male body size. The proportion of short morph sperm, however, did not influence sperm precedence even though it varied between 9% and 20% between the two males mating (Presgraves, 1997).

No evidence is available to evaluate whether short sperm aid in the transport or survival of long morph sperm. While short morph sperm seem unlikely to influence long morph survival in *Drosophila pseudoobscura* because they do not persist for more than two days in the female reproductive tract (Snook *et al*., 1994), similar data are not available for any diopsid. Short morph sperm potentially could influence long morph movement in several locations. Most male diopsids transfer sperm to females by creating a spermatophore, a gelatinous sac, inside the female (Kotrba, 1996). Sperm and accessory substances rapidly move out of the spermatophore and up the spermathecal ducts, and the female expels the spermatophore within an hour (Kotrba, 1993). For fertilization to occur, sperm must travel out of the spermathecae, down the spermathecal ducts, into chambers in the ventral receptacle and then successfully enter the micropyle of an egg when it is in position. Given the association between the length of the short morph sperm and the size of the spermatheca, closer examination of sperm movement in and out of this sperm storage organ could prove fruitful.

(4) Hemiptera: Pentatomidae

(*a*) *Distribution and development*

Regular occurrence of polymegaly in the spermatozoa of pentatomid stinkbugs is widespread in the subfamily Pentatominae (tribes Pentatomini, Halyini, Discocephalini, and Edessini), a group that comprises approximately four-fifths of the 3000 species in the subfamily (Bowen, 1922*b*; Schrader & Leuchtenberger, 1950). Polymegaly occurs in two distinct ways in the subfamily. In the most common form of polymegaly, spermatogenesis results in variation in sperm size but not in ploidy (number of chromosomes). However, in a subset of species, spermatogenesis results in sperm that vary in both size and ploidy. We discuss production of both sperm types in turn.

The testes of all species of Pentatomidae consist of a number of subdivisions, compartments or lobes (Fig. 4A shows the lobed testes of *Loxa flavicollis*, arbitrarily numbered 1–7 starting at the sperm duct). The most common number of lobes is seven, although there is variation among tribes and species (range = 3–8; Schrader, 1960*a*, *b*; Schrader & Leuchtenberger, 1950, 1951). Sperm characteristics (size and/or ploidy) differ among lobes of the testes.

First described by Montgomery in 1898 (cited in Schrader, 1960*b*), some species of pentatomid bugs produce spermatozoa that can be categorized into three main size classes, large, medium, and small. For example, in *Arvelius albopunctatus* the volume of the larger sperm size class is approximately eight times that of the smaller (Schrader & Leuchtenberger, 1950, 1951). In all described cases, the production of different size morphs of sperm is associated with particular lobes of the testes. Two of the lobes of the testes produce the larger sperm morph. These lobes flank the single lobe of the testes that produces the smaller sperm morph. The remaining lobes produce the medium-size gamete. It is important to note that these different size classes result from normal meioses. Thus, the protein and RNA content of the different sperm classes is positively related to size while the haploid chromosome number remains constant. Spermatocyte size is not correlated with the size of the lobe. In fact, the lobe that produces the smaller sperm morph is often the largest while the lobes that produce the largest sperm morph are the smallest (Schrader, 1960*a*, *b*; Schrader & Leuchtenberger, 1950, 1951).

The polymegaly described above differs quite dramatically from a second process seen in a small subset of species in the subfamily Pentominae, which

results not only in sperm of different size classes but also of different ploidy (Bowen, 1922*a*; Schrader, 1960*a*, *b*). Heteroploidy is not restricted to a monophyletic grouping of species but has been reported in the tribes Pentatomini, Halyini, and Discocephalini. As a result of an irregular meiotic process within a singe lobe of the testes, the number of chromosomes in developing spermatids ranges from one, which is always the sex chromosome, to over 100, more than 10 times the haploid number of chromosomes expected in the genus *Loxa* (Bowen, 1922*a*; Schrader, 1945*a*, *b*). This lobe was termed the ''harlequin'' lobe because deviations from the normal meiotic process are so striking. Interestingly, this lobe corresponds to the larger lobe that produces the ''small'' sperm morph in most of the species in the subfamily and becomes so disproportionately large, relative to the other lobes, that in some cases, it causes the testes to warp or coil. Schrader (1960*b*) noted that the physiological and chemical conditions within the ''harlequin'' lobe differ from those in the ''normal'' testes.

Despite this extraordinary variation in chromosome number, sperm development appears to proceed to completion in the harlequin lobe, resulting in sperm that vary in size depending on their chromosome content (Fig. 4B, C). Sperm size varies positively in relation to chromosome number. Even though sperm development proceeds regardless of chromosome number, current evidence indicates that only those sperm containing at least a full complement of chromosomes ever reach the sperm duct (Schrader, 1960*a*). Thus, the smallest sperm, which contain fewer than the normal complement of chromosomes, never leave the male.

(*b*) *Functional evidence*

Schrader (1945*a*, 1960*a*, *b*) wondered what evolutionary forces would lead to the persistence of the harlequin lobe, a structure that produces heteroploid sperm not used in fertilization. He suggested that they must provide additional nutrients, especially nucleoproteins, to the developing egg. As the harlequin sperm are probably not fertile, this argument is contingent on the occurrence of polyspermy. Neither the regular occurrence of polyspermy, in general, nor entry of the harlequin sperm into the egg, in particular, has ever been investigated in pentatomids. Although not noted by Schrader, heteroploid sperm might also serve other nutritive roles (i.e. to the female or to fertilizing sperm).

In a review of the occurrence of heteroploidy in Pentatomidae, Schrader (1960*b*) noted an apparent association with tropical and subtropical habitats for the 20 plus species (out of hundreds surveyed) that had the trait. No associations between the harlequin lobe and any ecological conditions have been discovered.

The widespread production of multiple sperm sizes that result from normal meiotic processes presents as much of an evolutionary mystery as the harlequin lobe. Even though many species of stinkbugs have polymegalous sperm that vary only in size but have a normal complement of chromosomes, all three sperm morphs may not participate equally in fertilization (see Snook *et al*., 1994). The fertilization competence of the different sperm morphs remains to be demonstrated. At least for the larger sperm morph, a similar nutritional argument as has been proposed for the larger ''harlequin'' sperm (Schrader, 1960*b*) could be advanced as a possible advantage for having multiple sperm morphs.

Research on the southern green stinkbug, *Nezara viridula*, provides some inferential support for a nutritive role of the larger 'harlequin' sperm morph. In paired mate choice tests, McLain (1998) found that males rejected as mates had smaller 'harlequin' lobes than did accepted males. Because females allowed to choose mates enjoyed higher reproductive success, measured as number of fertilized eggs produced during a lifetime, McLain (1998) inferred that females received non-genetic benefits from preferred males (see also McLain & Marsh, 1990). McLain (1998) suggested that a non-genetic benefit might accrue from giant, multinucleate sperm, if non-fertilizing sperm provide nutrients to eggs or females. For these arguments to hold, the width of the harlequin lobe must be correlated with the size or number of giant harlequin sperm produced, and, in the case of direct nutritional benefits to the egg, polyspermy must occur with some frequency. Across species, the width of the harlequin lobe does not predict size of sperm (Schrader, 1960*b*); within species the relationship between lobe width and either sperm size or number is unknown. Critical tests needed to evaluate whether heteromorphic sperm provide paternal contributions to the egg or to the female have yet to be done. Because both male attractiveness in this species, as well as in the green stink bug, *Acrosternum hilare* (see Capone, 1995), and male copulatory success is also correlated with male body size (McLain & Marsh, 1990) it is impossible to rule out simple body-size effects (e.g. larger spermatophore) on female productivity.

(5) Hymenoptera

Polymorphic sperm have been reported in the hymenopterous wasp *Dahlbominus fuscipennis* (Lee & Wilkes, 1965; Wilkes & Lee, 1965). In addition to noting some length variation, the authors found that the sperm have distinctive corkscrew-shaped heads but with gyres of two types, dextral or sinistral coils. Wilkes and Lee (1965) suggested that the distinctive morphs might influence sex ratio. *D. fuscipennis* is a haplo-diploid species that under a wide range of conditions produces approximately 90% female offspring (Wilkes, 1963). However, an inherited, sex-limited factor can lead to the production of 5% or fewer females (Wilkes, 1964). When the proportion of the two types of sperm in the spermatheca of females from strains of wasp that produce either female-biased or male-biased sex ratios is compared, a much lower proportion of dextrally coiled sperm were found in the male-biased strain (cited in Lee & Wilkes, 1965). They suggested that the sinistrally coiled sperm were not capable of penetrating the egg but can plug the micropyle and activate the egg for further development (Wilkes & Lee, 1965). As a result, the egg would develop into a haploid male wasp. We were unable to find any further published research to substantiate this particular hypothesis. The presence of spirally twisted spermatozoa has subsequently been reported in two other families of Hymenoptera, Eurytomidae and Pteromalidae (Hogge & King, 1975; Quicke *et al.*, 1992), but with no mention of a shape polymorphism (i.e. either coil orientation or length polymorphisms).

(6) Coleoptera: Carabidae

The presence of gigantic sperm has also been suggested to be widespread in the Carabidae (reviewed in Fain-Maurel, 1966) and has been reported for species in several genera including Acanthoscelides (Mulnard, 1951), Chrysocarabus, and Hadrocarabus (Bouix, 1961, 1963). The atypical sperm are always gigantic and hyperpyrene, including diploid, tetraploid, and higher orders of polyploidy (reaching chromosome complements of over 100 in some cases). In general, the morphology of the giant sperm is comparable to eupyrene spermatozoa, with the exception of the size of all constituent parts. However, in addition to more voluminous nuclei, giant sperm have been reported, in some cases, to be bi- or multinucleate. Also, in some species (e.g. *Acanthoscelides* spp.), the hyperpyrene sperm display differences other than increased volume, such as being shorter and thicker compared to eupyrene sperm (Mulnard, 1951). Aberration in chromosomal numbers appears to occur during the last gonial mitosis. Production of polyploid sperm in the Carabidae differs from what occurs in the Pentatomidae in which polyploid sperm production is localized to a specific lobe of the testes and in which only one nucleus has been reported (see above). No studies addressing the function of gigantic sperm in these species have been reported.

(7) Other insects

Gigantic sperm, similar to those seen in Pentatomidae, have also been reported for several groups of Orthoptera. In the cockroach, *Periplaneta americana*, giant sperm have been suggested to be the result of multinucleate, diploid, or higher degrees of heteroploidy (see Section III. 4; Richards, 1963). However, detailed work on neither spermatogenesis nor the function of heteromorphic sperm has been reported.

Phasmids, stick insects, have also been cited as exhibiting sperm size polymorphisms (e.g. Richards, 1963; Wilkes & Lee, 1965). The "numerous abnormal giant spermatozoa with three or four flagella" were the result of non-division of the spermatogonium; however, these results were reported for experimentally produced males of the normally parthenogenetic species, *Carasius morosus* (Bergerard, 1962), and, therefore, it is not clear whether this case is analogous to other cases of sperm heteromorphism described herein. This species rarely produces males, and it would be interesting to determine sperm morphology in these rare males.

Finally, a broad light microscopic survey over 50 European ephemeroptera, the most primitive extant pterygote insect, revealed a high level of structural diversity in sperm types (Soldan, 1979*a*, *b*), including aflagellate sperm. Polymorphic sperm that varied in size, shape, and stainability (Pappenheim's stain) were found commonly in six of the 11 families surveyed. No subsequent studies of sperm ultrastructure in ephemeroptera using more modern techniques have noted the presence of heteromorphic sperm.

IV. EVOLUTION OF SPERM HETEROMORPHISM

One of the most striking aspects of sperm heteromorphism is that some members of the ejaculate

appear to forgo opportunity for fertilization in *lieu*, presumably, of providing some benefit to the diploid male or ejaculate as a whole. How such a character would arise has been the subject of much speculation. Surprisingly, given that sperm competition is central to most of the current hypotheses concerning the function of heteromorphic sperm, little formal theory underlying such a trait has been developed. The idea that a 'helper' sperm type would evolve for the benefit of a second 'egg getter' type (Baker & Bellis, 1988) originally met with some criticism (Harcourt, 1989; Harvey & May, 1989) both because the fitness advantage of producing non-fertilizing sperm is unclear and because the persistence of such a phenotype would not necessarily be expected if each sperm behaved as an individual, with reproductive success dependent on the haploid genotype. Haploid determination of the sperm phenotype should lead to intraejaculate sperm competition (Parker & Begon, 1993; Sivinski, 1980). Such intraejaculate competition should then result in a conflict of interest between parent and gametes and should conspire to make evolution of cooperation within the ejaculate difficult (Haig & Bergstrom, 1995). Those sperm that did not cooperate and did not play a supporting role would be over-represented among the successful sperm in the male's ejaculate. Consequently, evolution most likely favours genes expressed in the male parent that suppress competition among sperm within a single ejaculate (Haig & Bergstrom, 1995).

Regarding sperm form and function, Sivinski (1980) pointed out the dual nature of sperm: each having a unique haploid genotype with the potential to express individual differences as well as being products of the male parent with function of sperm determined by the diploid genotype. Generally, the phenotypic characteristics of gametes appear to be established by the diploid parents. Sivinski (1980) suggested that haploid control was suppressed in animals to ameliorate the adverse effects of intraejaculate competition. However, haploid expression should not simply be dismissed as it has been shown to influence spermatogenesis and sperm function (Erickson, 1990). Selfish genetic elements such as meiotic drive or segregation distortion sex ratio systems (reviewed in Hatcher, 2000) provide one clear counter example in which haploid expression prevails. Haploid effects are also common in plants resulting in pollen competition (Delph & Havens, 1998). Haploid control may be more common in plants because plant population structure and pollen dispersal sufficiently reduce within-male pollen competition (analogous to intraejaculate competition) such that adverse effects from this source are minimal (Sivinski, 1980).

The weight of empirical evidence suggests that different morphological types of insect sperm are currently determined mainly by the diploid genotype. Where the mechanism is known, the signal for producing different sperm types comes from the male parent, in other words from the diploid genome. In cases where the exact mechanism is not known, indirect evidence indicates that the signals must emanate from outside the sperm, again indicating signals from the diploid genome. In Lepidoptera, the shift from producing only eupyrene sperm to producing only apyrene sperm is determined by an isolated circulating haemolymph factor, ASIF (Friedlander, 1997). In sperm heteromorphic *Drosophila* spp. and diopsid flies, all sperm within a single bundle have the same discrete morphotype, with little variation between individual sperm within a single bundle (Presgraves *et al*., 1997; Snook *et al*., 1994). In pentatomids, only sperm within particular lobes of the testes show variation in size. This is true regardless of the type of polymegaly displayed, e.g. either euploid sperm or polyploid, 'harlequin', sperm. Finally, the sperm produced by any individual male of the haplo-diploid wasp, *Dahlbominus fuscipennis*, are necessarily the same haplotype. Therefore, any differences between the various morphs (both coil direction and length variation) must be determined by signals coming from the male parent.

The fact that sperm heteromorphism is determined by the diploid parental genotype does not necessarily exclude a role for haploid control in the early divergence of the different sperm types. Parker and Begon (1993) showed that haploid control and intraejaculate competition can lead to different optima in size and number than predicted if sperm function is under diploid control. Theoretically, haploid control could contribute to subtle variation in morphs and therefore be important in the early evolution of the distinct morphs. However, precisely because there can be substantial differences in sperm haplotypes, Haig and Bergstrom (1995) suggested that evolution would favour suppression of competition within the ejaculate. They further postulated that size dimorphism would be more likely to evolve in haplo-diploid species because there is no genetic variation within the ejaculate. Conflict of interest between sperm and parent disappears because sperm and parent have the same genotype. *Dahlbominus fuscipennis* is the only haplo-diploid species in our review that shows within-male vari-

ation in sperm morphology. This may reflect sampling bias rather than true pattern. In order to satisfactorily test Haig and Bergstrom's (1995) hypothesis we would need a more systematic survey that focuses on the degree of sperm dimorphism in diploid versus haplo-diploid species. It would be interesting to survey sperm production more extensively in haplo-diploid genera such as ants, bees, and wasps.

In addition to wresting determination of sperm function from the haploid genome, emasculation of a sperm class to prevent offspring production has been suggested as a second means by which a male would be able to enforce division of labour and cooperation within his ejaculate (Haig & Bergstrom, 1995; Silberglied *et al.*, 1984). The apyrene sperm of Lepidoptera and the 'harlequin' sperm of some Pentatomidae are incapable of producing viable offspring because they either lack nuclei or possess an aneuploid number of chromosomes. In some cases where both sperm types contain a euploid number of chromosomes, the available evidence, which was reviewed above, suggests that only one of the typical sperm morphs ever participates in fertilization.

Recently, the conditions required to evolve parasperm were formally modeled (Kura & Nakashima, 2000). Because the function of parasperm has yet to be definitively established, Kura and Nakashima (2000) assumed that parasperm function as a caste of 'soldiers' adapted to eliminate rival sperm. Some key assumptions of their models are as follows: (1) competition is between sperm from two rival, unrelated males, (2) numerical superiority was determined randomly across a range of variation in sperm number, (3) soldier sperm kill only rival male sperm (i.e. perfect recognition of self), (4) there is no cost to producing either reproductive or soldier sperm, (5) sperm interact (destroy rival sperm) prior to opportunities for fertilization, and (6) the proportion of sperm types for each male is unchanged before and after interaction (i.e. soldier sperm destroy both rival sperm morphs equally). Models were tested assuming both diploid and haploid determination of sperm function (i.e. the level of sperm-male conflict was allowed to vary).

The way in which Kura and Nakashima (2000) defined the role of the 'soldier' sperm (i.e. one parasperm interacts with and kills an average number of rival sperm) best fits the elimination hypothesis but does not fit any of the other hypotheses related to the function of parasperm (i.e. blocking, cheap filler, sperm provisioning, sperm facilitation). The main conclusions from their analyses were as follows: (1) a second class of sperm within the ejaculate could evolve even if each 'soldier' sperm functionally removed only one or less than one rival eusperm from competition, and (2) under diploid control of sperm function, the ratio of parasperm to eusperm should increase until the ability of the remaining eusperm to fertilize all available ova was compromised.

Their analysis shows that the most favorable conditions for the evolution of 'soldier' sperm occurred when variance in the number of competing sperm was large (Kura & Nakashima, 2000). 'Soldier' sperm were particularly useful in conjunction with sperm displacement because they could then effectively eliminate the few remaining rival sperm. This finding is in agreement with the conditions that probably face sperm in competition. Variation in timing of copulation, sperm displacement, variation in ejaculate size, differential storage and usage, to name just a few factors, would all conspire to make the likelihood of equal representation between two or more ejaculates remote.

The final subsidiary finding revealed by Kura and Nakashima's (2000) models was that parasperm were less likely to evolve if sperm-male conflict, i.e. haploid determination of function, was allowed. If sperm were allowed to determine their own specialization, then the fitness advantage (indexed by the number of rival sperm disabled) had to be greater for the number of parasperm to increase. Furthermore, the ratio of parasperm to eusperm could not increase to the same level as with diploid control but would reach an optimum at a lower ratio that reflected the intensity of conflict between sperm and parent interests. Based on these results, they suggest that the evolution of specialized classes of sperm should be more easily accomplished in haplo-diploid organisms in which sperm-male conflict is not possible.

V. FUTURE DIRECTIONS

The origins of heteromorphic sperm appear to have deep phylogenetic roots. For example, sperm heteromorphism in *Drosophila* has an ancient and single origin in the common ancestor to all species in the *obscura* group. Similarly, dichotomous sperm production has been described for every species, except two from the most basal family (Micropterigidae), in the order Lepidoptera (Sonnenschein & Hauser, 1990). Although variation in characteristics of parasperm production (i.e. length, proportion, etc.) between species within these groups suggests that the

function of parasperm is also shaped by more recent selective pressures facing each species, phylogenetic analyses have enjoyed only limited success in shedding light on the role of parasperm (e.g. Snook, 1997).

The ancient evolutionary origins of polymorphic sperm have probably contributed to the difficulty in exposing their function. Due to phylogenetic history, comparisons of closely related sperm-monomorphic versus sperm-heteromorphic species have, in most cases, been impossible. Unfortunately, it is such sister taxa comparisons that will likely be most informative for unraveling the function of heteromorphic sperm. Future comparative studies focusing on groups with multiple gains and losses of sperm heteromorphism are warranted. For example, in Lepidoptera focusing on the primitive suborders might be enlightening because there is evidence for at least two independent evolutionary origins of sperm heteromorphism in the basal Lepidopteran families Micropterigidae and Agathiphagidae (Sonnenschein & Hauser, 1990). Additionally, sperm heteromorphism has not been investigated in either Heterobathmiidae or Eriocraniidae, two other primitive Lepidopteran families. Stalk-eyed flies also warrant further comparative study since there has been at least one loss of dimorphism in the family. Furthermore, because sperm dimorphism represents the ancestral state in the family, sperm production of closely related families should also be investigated.

From a comparative perspective, Pentatomidae and Carabidae may prove the most fruitful for deciphering the function of heteromorphic sperm. Although euploid polymegaly is widespread in Pentatomidae (subfamily Pentatominae), the presence of the harlequin lobe and the production of polyploid sperm must have evolved separately, multiple times in the tribes Pentatmomini, Halyini, and Discocephalini (Schrader, 1960*b*). The production of polyploid sperm has evolved independently in the Carabidae. The distribution of polymegaly in this group is the most poorly known of any insect group described herein, but it potentially contains sister taxa that differ in sperm production and would provide an independent sample that could be used to test Schrader's (1960*b*) original provisioning hypothesis for the function of giant, polypoid sperm. Furthermore, it appears that the presence of sperm heteromorphism has not been investigated in any systematic or thorough way not only in Carabidae but also in insects in general. It is quite possible that additional groups of insects that would be amenable to comparative analyses, i.e. groups with multiple independent evolutionary shifts towards sperm heteromorphism, have yet to be discovered.

Even within groups, comparative studies might prove informative, but a shift in focus to alternative characters may be necessary. Comparative studies to date have focused on species differences in length of the short and long sperm morphs (e.g. Gage, 1994; Morrow & Gage, 2000; Snook, 1997) and associations with degree of polyandry. However, gamete size is just one of several characters that might be important in the evolution of insect mating systems that involve multiple sperm types: age at reproductive maturity, ejaculate investment (including gamete size, gamete number, proportion of parasperm, seminal nutrition), remating frequency, duration of copulation, and body size are some other mating system features that would be worth examining in a phylogenetic context (Markow, 1996). Integrating the results from multiple comparative studies might provide additional power to determine the functional significance of parasperm.

For example, the number of parasperm produced in the testes or delivered in the ejaculate might provide more insight into their function than length alone. To begin with, we have quantitative predictions for how parasperm numbers should change under the assumptions of at least one hypothesis. Kura and Nakamura (2000) predict that, under the "elimination" hypothesis, parasperm number should increase until the ability of eusperm to fertilize all available eggs is compromised. Similar models using assumptions that more closely match the behavior of sperm under the different hypotheses (e.g., blocking, elimination, provisioning, facilitation) should be generated.

In Table 2, we have compiled data on the proportion of parasperm produced and sperm lengths in the Diptera and Lepidoptera. Sperm length data for many more lepidopteran species are available elsewhere (Gage, 1994; Morrow & Gage, 2000), but because data on per cent apyrene sperm were not available they are not presented here. Inspection of the data (Fig. 5) reveals extensive variation between species in the proportion of parasperm produced, but that, in general, stalk-eyed flies produce the lowest percentage parasperm while lepidopterans produce the highest and *Drosophila* subgroup *obscura* spp. are intermediate (Fig. 5). The production of heteromorphic sperm arose independently in these three clades. If this was in response to the same selective pressure (e.g. sperm competition) then it appears that the evolutionary solution to the

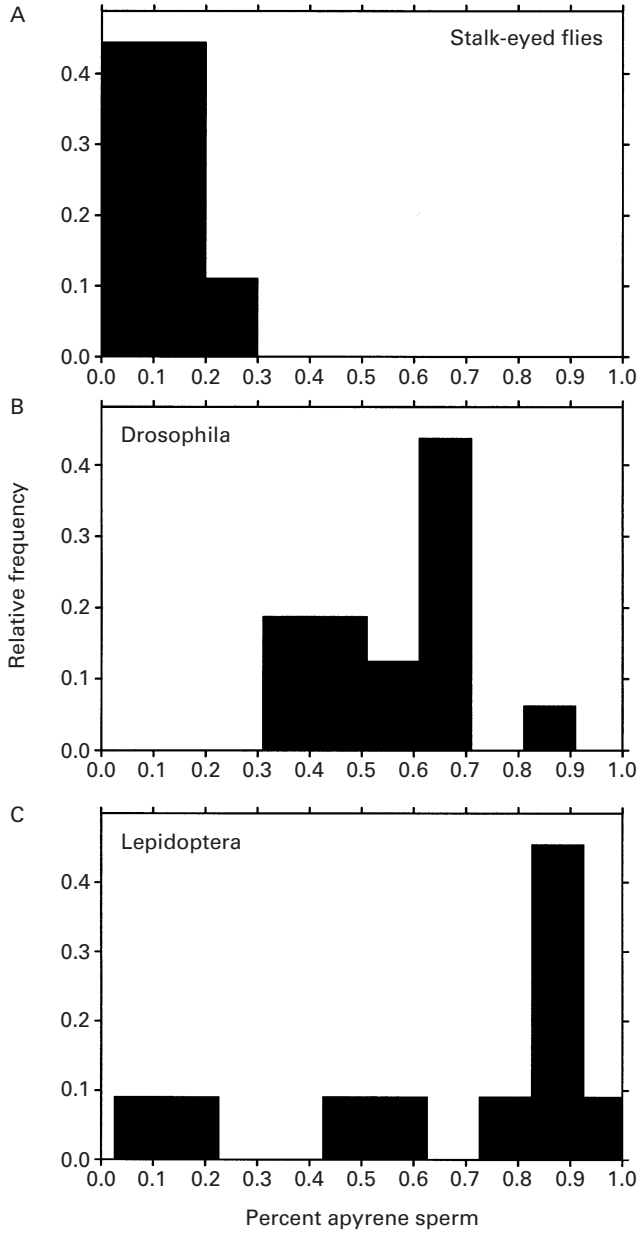

Fig. 5. Frequency distribution of per cent parasperm in stalk-eyed flies (A), *Drosophila obscura* group (B), and Lepidoptera (C).

problem may have been slightly different in each case. In stalk-eyed flies, only one out of nine species surveyed had more than 20% parasperm (Table 2; *Teleopsis quadriguttata*, 24% parasperm). Given the low proportion of parasperm produced, it seems unlikely that they serve as "cheap filler". In Lepidoptera, the situation is reversed; of 22 species for which we have sperm count data, 13 have greater than 80% parasperm. Thus, for most species of moths and butterflies, the "cheap filler" hypothesis remains a likely candidate. Interestingly, four lepidopteran species are reported to have less than

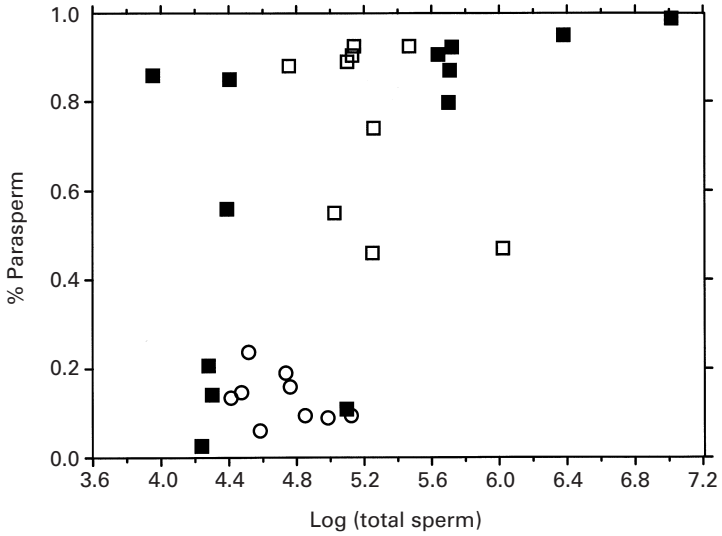

Fig. 6. Plot of per cent parasperm *versus* total sperm count (log-transformed) for stalk-eyed flies (open circles) and Lepidoptera (squares). Sperm counts in Lepidoptera were either from the male seminal vesicle (filled) or from spermatophores in the female bursa copulatrix (open).

20% parasperm. There is the possibility that parasperm perform multiple functions, as do eusperm in sperm-monomorphic groups (e.g. Baker & Bellis, 1988, 1989), and depending on the species one function may predominate.

The number of parasperm and or the proportion of parasperm produced could be viewed as a rough index of the energetic investment in parasperm, assuming that each sperm costs some finite amount of energy or resources to produce. Inspection of Shephard's unpublished data (Table 2) on sperm counts from the seminal vesicles of male Lepidoptera shows an asymptotic relationship between the proportion of apyrene sperm in the ejaculate and total sperm numbers (log-transformed; Fig. 6). The same general relationship holds if sperm count data from the remaining Lepidoptera, which were generated from spermatophore counts, are included. These data are generally consistent with either the "elimination" or the "cheap filler" hypothesis. No relationship exists between these two variables in stalk-eyed flies (Fig. 6). In Drosophilidae, sufficient data to assess this relationship do not exist.

Data on sperm counts may even be able to speak to the cost of producing parasperm. That parasperm are energetically inexpensive to produce is one of the underlying assumptions of the "cheap filler" hypothesis. If only the energetic cost of sperm production determines the proportion of the ejaculate dedicated to each sperm type this might be reflected in the numerical relationship between parasperm

and eusperm. If the energetic cost of producing the different sperm types does not differ then the relationship between log number parasperm and eusperm should be linear with a slope of 1 (Fig. 7). A slope greater than 1 would indicate that short sperm are less expensive to produce, since for every incremental increase in long sperm a proportionally larger increase in short sperm would be realized. A slope less than 1 would indicate the reverse, that short sperm are more expensive to produce (Fig. 7). Plotting log parasperm number *versus* log eusperm number data for Lepidoptera and stalk-eyed flies separately shows that these data are not consistent with energetic cost being the only factor driving the composition of the ejaculate (Fig. 7). The slope of the relationship is does not differ from unity in either lepidopterans (Slope $\pm$ S.E. $= 1.159 \pm 0.52$) or stalk-eyed flies (Slope $\pm$ S.E. $= 0.598 \pm 0.28$). Based on these data, there is no compelling evidence that the energetic production costs of short sperm differs significantly from those of long sperm.

Experimental studies that manipulate sperm proportions provide a complementary means of disentangling parasperm function that has been underutilized. Particularly for groups where the potential for sister taxa comparisons is limited, an experimental approach may prove the most informative. In Lepidoptera, a variety of experimental methods have been used to alter the spermatophore: irradiation, nutritional stress, and mating status. Irradiation had the undesired side effect of developmentally altering eusperm (Richard *et al.*, 1975). Mating status did alter the composition of apyrene and eupyrene sperm (Wedell & Cook, 1999*b*) but, like nutritional stress (Gage & Cook, 1994), reduced spermatophore size (Cook & Wedell, 1999; Wedell & Cook, 1999*b*). A variety of other means of manipulating spermatophore size remain untapped. For instance, it may be possible to exploit individual and population variation in ejaculate composition. For example, Presgraves (1997) has shown a difference in per cent parasperm between two populations of a stalk-eyed fly, *Cyrtodiopsis whitei*. Experiments using alternate matings of males from these divergent populations might shed light on the function of short sperm in stalk-eyed flies. Second, if individual variation in parasperm has an additive genetic component then artificial selection could be employed to create lines that differ in sperm composition (e.g. Wilkes, 1964). Finally, it may be possible to alter the development of the different sperm types. For example, in Lepidoptera if the action of ASIF (Friedlander, 1997) could either be

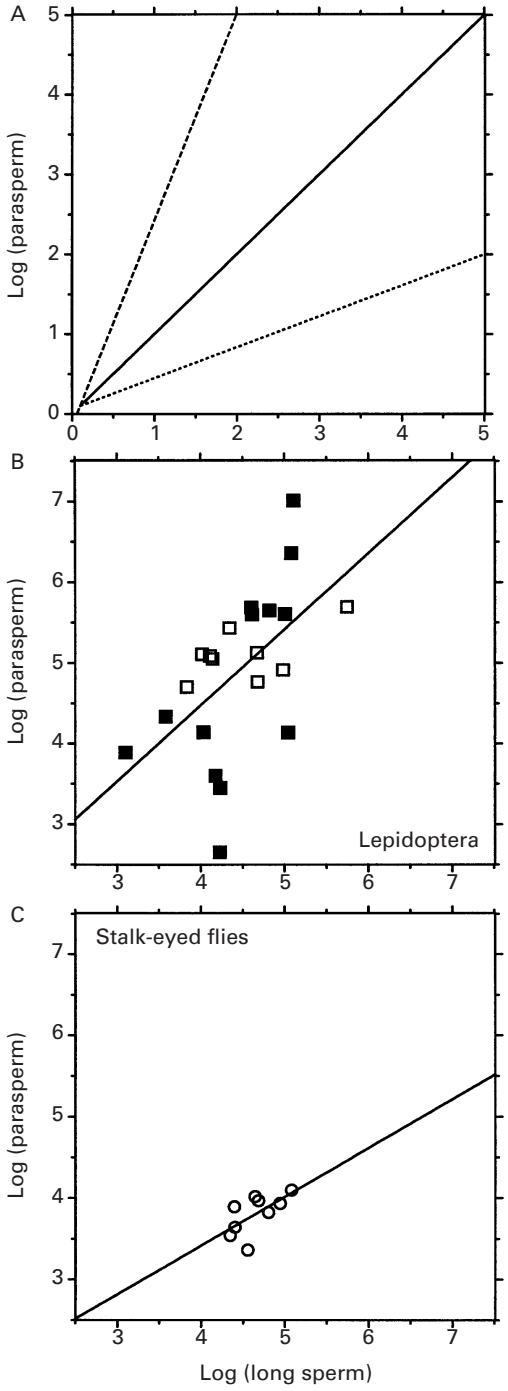

Fig. 7. Plots of number of parasperm (log-transformed) *versus* number of long sperm (log-transformed). (A) A line with a slope of 1 is the relationship expected if the number of each sperm type is a function solely of the energetic cost of producing sperm and when the cost of producing each sperm type is equal. A steeper relationship (i.e. slope > 1) between parasperm and eusperm would indicate that parasperm are less expensive to produce; a more shallow relationship (i.e. slope < 1) would indicate that parasperm are more expensive to produce. (B, C) The relationship is plotted for Lepidoptera (B) and stalk-eyed flies (C). The lepidopteran data are split by the method of

greatly reduced (e.g. knocked out) or stimulated it might be possible to influence the proportion of different sperm produced without influencing overall spermatophore size. Finally, lepidopteran sperm have been successfully fractionated, isolating eupyrene sperm bundles and apyrene spermatozoa from seminal fluid, for physiological studies of capacitation (Osanai & Isono, 1997; Osanai, Kasuga & Aigaki, 1989*b*). Using such methods, it may be feasible to artificially inseminate females with sperm that vary from 0–100% parasperm It would be worthwhile to extend such methodology to other groups that exhibit dichotomous sperm production as well.

VI. CONCLUSIONS

(1) Interest in the evolution of heteromorphic sperm remains high despite the difficulty of explicating their significance. To date, no conclusive evidence exists for any of the current hypotheses concerning the function of heteromorphic sperm (Table 1), even in the two most extensively studied groups, Lepidoptera and species of *Drosophila*. From our review of the literature, we find no reason to believe that heteromorphic sperm perform the same function in different groups of organisms. Sperm heteromorphism has arisen independently in multiple groups. Variation in parasperm morphology is extensive between these groups, not to mention variation in mechanism and amount of production.

(2) Comparative analyses have had only limited success unraveling the function of parasperm. Such analyses provide weak support for the ''cheap filler'' hypothesis in Lepidoptera (Gage, 1994; Morrow & Gage, 2000). In stalk-eyed flies, Presgraves *et al.* (1999) suggest that short-sperm function must localize to the sperm storage organs and that spermathecae are important agents of selection on short sperm. These results are consistent with any of the sperm competition hypotheses, but the proportion of short-sperm and unpublished experimental studies suggest that the ''blocking'' hypothesis is the most likely explanation of their function (Presgraves, 1997). In *Drosophila* spp., phylogenetic analyses have not been able to provide support for any of the hypotheses discussed in this review but do suggest that the selective pressures on short and long sperm are decoupled (Snook, 1997). Similar phylogenetic analyses have not been undertaken for any of the other groups of species discussed in this review. Some of the difficulty resolving the function of parasperm with comparative studies probably arose because of the deep phylogenetic roots from a single common ancestor. We suggest two ways of improving future phylogenetic studies. First, we recommend that groups with multiple gains and losses in sperm heteromorphism should be studied in order to increase the number of sister taxa that differ in sperm production. Second, we propose that subsequent comparative analyses should focus on more characters than simply gamete size.

(3) We advocate a larger role for experimental studies in the future. The strongest evidence for the function of apyrene sperm comes from an experimental study (Cook & Wedell, 1999). Taking advantage of natural variation in proportion of each type of sperm produced or experimentally altering the composition of a male's ejaculate in order to manipulate the amount of each sperm type a female receives is more likely to provide more illuminating information regarding the function of parasperm than comparative studies.

sperm counting: counts from the seminal vesicles (filled squares) and counts from spermatophores in the bursa copulatrix (open squares). The slope of the relationship is slightly greater than 1 for lepidoterans (Slope $\pm$ s.e. = 1.159 ± 0.52) and slightly less than 1 for stalk-eyed flies (Slope $\pm$ s.e. = 0.598 ± 0.28), but the deviation from unity is not statistically significant in either group.

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