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Original article

Variation of sperm length and heteromorphism in drosophilid species

D. Joly, M.-L. Cariou, D. Lachaise and J.R. David

Centre National de la Recherche Scientifique, laboratoire de biologie et génétique évolutives, 91198 Gif-sur-Yvette Cedex, France

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Summary – Sperm length was measured in 27 drosophilid species, and a general survey of size variation is presented for 75 species for which information is available. Mean length varies from 0.113 mm in the *D. obscura* group to almost 20 mm in the recently investigated *D. littoralis;* in the latter case, sperm length is nearly 6 times the male body length. The huge interspecific variability may be estimated by considering the coefficient of variation (c.v.) between species belonging to the same taxon. In the genus *Drosophila* the c.v. amounts to 130% (64 species). The average c.v. decreases in lower taxa, being for example 96% in subgenera and 61% in species groups. More closely related species are thus less divergent, but in any case sperm length must be considered as a fast evolving trait, increasing or decreasing. Individual measurements of sperm length within a species generally provide a unimonal, relatively gaussian distribution (monomorphism). By contrast, 13 investigated species of the *D. obscura* group exhibited bimodal distributions. This heteromorphism may be considered as a stable evolutionary strategy in the *D. obscura* group.

***Drosophila* – sperm length – sperm heteromorphism – sperm evolution**

Résumé – Variation de la longueur des spermatozoïdes et hétéromorphisme chez les drosophilidés. *La longueur des spermatozoïdes mesurés chez 27 espèces de Drosophilidés est étudiée au niveau de l'ensemble de la famille (75 espèces). Les moyennes de longueur varient de 0,113 mm chez les espèces du groupes* obscura *jusqu'à 20 mm chez une espèce nouvellement étudiée,* D. littoralis; *chez cette dernière, la longueur du spermatozoïde atteint 6 fois la longueur du corps de l'adulte. L'amplitude de la variation interspécifique peut être appréciée en considérant le coefficient de variation (c.v.) entre espèces appartenant à un même taxon. Dans le genre* Drosophila *(64 espèces étudiées), la valeur du c.v. atteint 130%. Les valeurs des c.v. moyens diminuent pour les niveaux taxonomiques inférieurs, c'est-à-dire 96% au niveau du sous-genre et 61% pour les groupes d'espèces. Les longueurs de spermatozoïdes des espèces étroitement apparentées sont relativement proches mais dans quelques cas, il apparaît que ce caractère évolue rapidement, tendant, soit à augmenter, soit à diminuer. Les mesures de la longueur des spermatozoïdes pour une espèce correspondent généralement pour un individu à une distribution unimodale, gaussienne (monomorphisme). En revanche, les 13 espèces du groupe* obscura *montrent des distributions bimodales. Cet hétéromorphisme peut être considéré comme une stratégie évolutive stable.*

Drosophile – *longueur des spermatozoïdes* – *hétéromorphisme des spermatozoïdes* – *évolution des spermatozoïdes*

INTRODUCTION

In most Eucaryote species, meiotic reproduction has evolved in producing two sizes of gametes, known as the macro, or female gamete (oocyte or ovum) and the micro, or male gamete (sperm) respectively (Parker *et al.*, 1972; Power, 1976; Maynard Smith, 1978; Alexander & Borgia, 1979; Parker, 1984). In the macrogamete, size variations between taxa are well documented and incorporated in the evolutionary theories of parental investment (Trivers, 1972) and life history strategies (Throckmorton, 1966).

By comparison, evolutionary trends in the microgamete (sperm) have remained neglected. Indeed, when considering vertebrates an overall uniformity seems the rule, yet some differences in sperm heads or tails have been found in rabbits and rodents (Friend, 1936; Beatty & Napier, 1960; Beatty & Sharma, 1960; Woolley, 1971). In these, sperm with a medium size flagellum less than 0.1 mm long are produced in huge numbers and each gamete has an extremely low probability of producing a zygote. Sperm shape, size and ultrastructure are, however, far more diverse among invertebrates (Fain-Maurel, 1966; Afzelius *et al.*, 1976; Baccetti, 1979; Sivinski, 1984; Chauvin *et al.*, 1988) and analysing this diversity should help to understand the developmental constraints and selective pressures which permitted or promoted the various patterns presently observed. In some invertebrates, including Lepidoptera, two or more morphologically and functionally different microgametes occur intraspecifically and warrant recognition of eusperm and parasperm (Healy & Jamieson, 1981; Jamieson, 1987a).

In the present work, attention is focused on the evolution of sperm length in a monophyletic dipteran taxon, the family Drosophilidae. *Drosophila melanogaster,* generally considered as a reference for this group, is known for its long sperm (1.9 mm) which is almost as long as the body of the fly (Cooper, 1950; Yanders & Perras, 1960; Beatty & Burgoyne, 1971; Gould-Somerot *et al.*, 1974; Joly, 1987). However, at the family level, *D. melanogaster* sperm can appear very short compared to those of other species such as for instance *D. hydei,* where it can be 4-5 times (1.4 or even 1.9 cm) the size of the body of the fly (Hess & Meyer, 1963; Jamieson, 1987b).

Taxonomists have long been aware that, in the family Drosophilidae, sperm length could be very variable between species and several papers have been recently devoted to this problem (Beatty & Sidhu, 1970; Sanger & Miller, 1973; Gromko *et al.*, 1984; Sivinski, 1984; Hatsumi & Wakahama, 1986; Hihara & Kurokawa, 1987; Joly, 1987). Explaining such variations raises an evolutionary challenge which may be formulated as follows: if sperm length is a fast evolving trait, it should exhibit a high genetic variance and a high heritability, at least in some species; moreover, a rapid evolution for increased or decreased length would be difficult to explain if the trait is considered as neutral, and strong selective pressures should exist or have existed during the process of speciation.

In *Drosophila,* intraspecific genetic variability of sperm length is poorly documented and presently available investigations have failed to demonstrate genetic variance (Joly, 1987) or have found only very limited variability (Beatty & Sidhu, 1970; Sanger & Miller, 1973). The aim of this paper is to focus attention on interspecific variations and to present an overview of what is known in the family.

In the genus *Drosophila,* sperm length can indeed be considered as a fast evolving trait with a loose relationship with phylogeny. In most species, sperm length distributions within the individual are unimodal with a limited variability. However, in one monophyletic taxon, the *D. obscura* species group, the occurrence of bimodal distributions seems the rule and we therefore argue here that it corresponds to an evolutionary stable strategy (ESS) (Maynard Smith, 1974).

MATERIAL AND METHODS

Sperm length was measured in 27 species, 13 of which belong to the *obscura* species group including the two recently discovered East African species *(D. microlabis* and *D. kitumensis)* (Cariou *et al.,* 1988). The source of material of the *obscura* group species investigated was the same as in Cariou *et al.* (1988) with the exception of *D. affinis* (14012, 014-1) and *D. azteca* (14012-0171) which were provided by the Bowling Green Stock.

The eight species of the *melanogaster* subgroup were analyzed. The source of specimens of the *melanogaster* complex species was the same as in Joly (1987) while those of the others were the following: *D. teissieri* and *D. yakuba* came from different localities in Africa (Gif Stock); *D. erecta* (Ivory Coast, Gif 220-5) and *D. orena* (West Cameroon, Gif 188-1).

Other drosophilid species studied were *D. bahunde* (Kenya, Gif 269-4), *D. bakundjo* (Kenya, Gif 269-5), *Scaptomyza pallida* (Kenya, Gif 292-2), *Zaprionus tuberculatus* (West-Africa), *D. grimshawi* (Hawaii) and *D. littoralis* (unknown Palearctic origin) from the Bowling Green Stock.

The strains were reared at 21 °C. Sperm were recovered from the seminal vesicles of one or several males. The testes were isolated and opened in a drop of saline solution and the sperm allowed to spread out. This preparation was observed under a microscope with phase contrast optics. When the sperm had ceased to move, they were traced with the aid of a camera lucida and the trace lengths measured with a cursor on a digitizing table connected to a microcomputer. Except for the *obscura* group species, the measure of cyst length was preferred to that of sperm length to minimise the risk of breakage. All details of this method are given in Joly (1987 and 1989).

The sperm length of the 48 other species belonging to different taxa of the Drosophilidae are provided in the literature (Sanger & Miller, 1973; Hatsumi & Wakahama, 1986; Hihara & Kurokawa, 1987).

RESULTS

Results for the investigated species are given in Table I and for the 13 species of the *D. obscura* group in Table III. Some of the species presented in these tables have already been studied by other investigators, for example *D. melanogaster* (Table IV) and some species in the *D. obscura* group. Our measurements are, on the whole, in good agreement with previous data in spite of methodological problems mainly due to the difficulty in obtaining identifiable and unbroken cells. It is therefore possible to present (Table II) a general overview of size variability across the entire Drosophilidae family.

Table I. Biometrical data of sperm length (cysts) for species investigated other than those of the *D. obscura* group.

Species	*n*	*m*	*s.e.*	*c.v.*
Drosophila SG Sophophora				
D. melanogaster	150	1.898	.008	5.3
D. simulans	350	1.124	.002	4.3
D. mauritiana	200	1.036	.004	5.7
D. sechellia	200	1.649	.008	7.7
D. yakuba	1200	1.681	.008	13.5
D. teissieri	1300	1.606	.008	19.9
D. erecta	50	1.210	.004	2.4
D. orena	50	1.436	.006	3.4
D. bahunde	1	3.144	–	–
D. bakundjo	1	3.173	–	–
Drosophila s. str.				
D. littoralis	1	19.297	–	–
D. grimshawi	57	1.664	.011	5.1
Zaprionus tuberculatus	8	3.729	.218	16.6
Scaptomyza pallida	17	1.257	.011	3.6

n = number of sperm measured; m = mean of sperm length in millimeters; *s.e.* = standard error; *c.v.* = coefficient of variation.

At a genus level, mean length varies from 0.63 *(Amiota)* to 5.32 mm *(Mycodrosophila)*. However, among the 75 species presently studied, 64 belong to the *Drosophila* genus which is itself characterized by a huge interspecific heterogeneity. A more detailed analysis according to taxonomic subdivisions is presented in the lower part of Table II. The best documented subgenera, *Drosophila* and *Sophophora,* exhibit significant sperm length variations, with means of 5.03 and 1.14 mm respectively. Also, within each subgenus, lower taxa, *i.e.* species groups, may have different lengths and variations. For example, in *Drosophila,* flies in the *D. immigrans* group have much shorter sperm than in both the *D. repleta* and *D. virilis* groups which are characterized by very long sperm; the record length is provided here by *D. littoralis* from the latter group where it reaches 2 cm, that is 6 times the body length (Table I). In *Sophophora,* we may further contrast the *D. melanogaster* and the *D. obscura* species groups with means of 1.45 and 0.30 mm respectively.

Another way of analysing the data is to consider the heterogeneity among species belonging to taxa of similar levels. Since mean lengths are so variable, variances cannot be used directly and a relative measure, the coefficient of variation (c.v.) has therefore been preferred. This analysis is limited to *Drosophila,* since other genera are poorly documented. On the other hand, the *Drosophila* genus comprises so many species (over 1 500) that taxonomists felt the need for a series of hierarchical subdivisions, as defined, for example, by Bock & Wheeler (1972)

Table II. Biometrical data for different taxa, genus (G), subgenus (SG), group (gr.) and subgroup (sgr.).

	N	*m*	*s.e.*	*c.v.*	*range* *min*	*max*
Genera of Drosophilidae						
G. Amiota	2	0.63	0.01	1.1	0.63	0.64
G. Leucophenga	3	1.20	0.09	14.7	1.08	1.38
G. Drosophila	64	2.50	0.40	130.2	0.11	19.00
G. Liodrosophila	1	2.47	–	–	–	–
G. Mycrodrosophila	1	1.06	–	–	–	–
G. Mycodrosophila	1	5.32	–	–	–	–
G. Scaptomyza	2	0.92	0.34	52.0	0.58	1.25
G. Zaprionus	1	3.73	–	–		
Genus Drosophila						
SG. Dorsilopha	1	1.10	–	–	–	–
SG. Drosophila	22	5.03	0.93	87.2	1.27	19.00
immigrans gr.	10	2.67	0.39	47.0	1.27	5.06
nasuta sgr.	8	2.85	0.46	45.9	1.27	5.06
quinaria gr.	2	3.36	2.37	20.6	2.87	3.85
repleta gr.	2	12.00	2.00	23.6	10.00	14.00
robusta gr.	2	5.36	3.09	2.0	5.29	5.44
virilis gr.	2	12.70	6.26	69.8	6.47	19.00
SG. Hirtodrosophila	3	2.06	0.85	71.7	0.36	3.04
SG. Scaptodrosophila	4	0.86	0.41	96.4	0.21	2.02
SG. Sophophora	34	1.14	0.20	103.5	0.12	5.37
dentissima gr.	2	3.15	0.05	2.2	3.10	3.20
melanogaster gr.	14	1.93	0.28	55.6	1.00	5.37
melanogaster sgr.	8	1.45	0.12	23.6	1.00	1.90
montium sgr.	2	3.79	1.57	58.8	2.22	5.37
obscura gr.	18	0.30	0.06	95.8	0.11	1.07
affinis sgr.	7	0.53	0.13	68.1	0.17	1.07
microlabis sgr.	2	0.13	0.01	11.3	0.12	0.14
obscura sgr.	7	0.15	0.01	20.1	0.11	0.20
pseudoobscura sgr.	2	0.16	0.01	4.3	0.16	0.17

N = number of species; *min* = smaller sperm length values; *max* = higher sperm length values in millimeters. Other abbreviations as in Table I.

who recognized subgenera, species groups, species subgroups, and within the latter, species complexes, species "clusters" , and pairs or groups of sibling species.

At the genus level, the overall c.v. is 130% (Table II) which means that the standard deviation is higher than the mean and the actual distribution is strongly skewed towards high values. Considering lower level taxa leads to lower values of c.v., *i.e.* 96%, 61% and 34% respectively for subgenera, species groups and species subgroups. It appears that homogeneity increases when more closely related species are compared.

It has been shown that intraspecific genetic variability in sperm length is poorly documented in *Drosophila* and requires further investigation. However, within each

species, the shape of the distributions of individual sperm measurements is worthy of consideration, and examples of such distributions are given in Figure 1. In *D. melanogaster* and *D. simulans* the distributions are obviously unimodal and close to a gaussian curve. Such is not the case in species of the *D. obscura* group, which exhibit clear-cut disjoint distributions. This intraspecific and intraindividual heterogeneity was already known in some of these species and the word *polymegaly,* meaning several sizes, was coined to describe this situation (Beatty & Sidhu, 1970; Beatty & Burgoyne, 1971). Our results confirm and extend these observations. In some cases, such as *D. pseudoobscura,* it could be argued that, by visual inspection, several peaks may be recognized. However, no statistical method exists for counting the number of peaks in a distribution. On the other hand, visual inspection always shows a well defined peak for short sperm while the situation may be more complex for longer sperm. As a conservative measure, it was decided to differentiate only two size classes in each species, *i.e.* short and long sperm, the size limit between the two classes being in most cases easy to define. Morphometric data, analysed in this way, are presented in Table III for the 13 investigated species of the *D obscura* group.

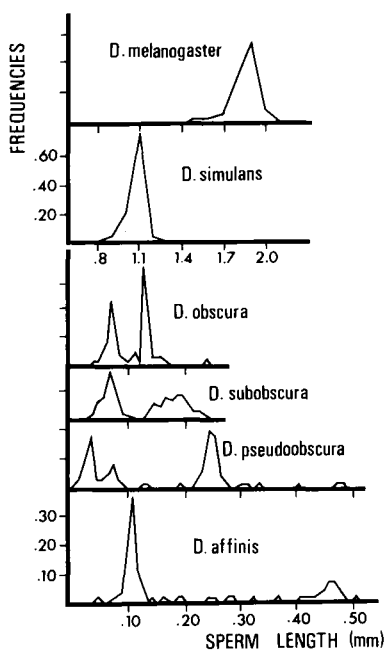

Fig. 1. Sperm length distributions for two sibling species of the *D. melanogaster* subgroup (above) and for four species of the *D. obscura* group. Note the different scale between the two taxa.

The shorter sperm class is variable in proportion from 30 to 88% of the distribution, but the average value is close to 50%. In several species, *e.g. D. pseudoobscura,* the short sperm has a length of about 0.050 mm, close to the usual

size of mammals. The interspecific variation ranges between 0.056 and 0.143 mm. By contrast, the long sperm class is more variable, ranging from 0.139 to 0.925 mm, and is also more heterogenous, as shown by its high c.v. When the two classes are pooled, the bimodality of the distributions is evidenced by the very high c.v. : 53%.

Table III. Biometrical data for short and long sperm classes within the D. obscura species group.

SPECIES			*shorter sperm*			*longer sperm*		*Total*		*Range*	
	n	*%*	*m*	*c.v.*	*limit*	*m*	*c.v.*	*m*	*c.v.*	*min*	*max*
affinis subgroup											
D. azteca	97	88	143	12.6	202	925	41.9	240	121.0	102	1339
D. affinis	305	69	112	7.1	131	424	21.5	177	79.6	53	535
D. helvetica	356	42	100	12.0	139	223	10.3	170	37.5	44	262
microlabis subgroup											
D. kitumensis	200	70	87	10.3	103	248	14.9	135	56.8	52	319
D. microlabis	200	62	68	14.7	104	196	14.3	115	56.3	33	263
obscura subgroup											
D. bifasciata	200	30	83	19.3	123	228	12.3	185	38.2	44	293
D. guanche	200	49	131	7.6	157	273	9.5	202	26.4	107	344
D. madeirensis	200	64	137	10.2	167	218	11.0	166	26.0	81	284
D. obscura	83	42	76	11.8	112	139	13.7	113	31.2	40	170
D. subobscura	210	48	85	15.3	128	199	12.6	142	42.3	50	255
D. tristis	200	64	112	10.7	147	235	8.9	152	39.8	55	274
pseudoobscura subgroup											
D. persimilis	200	50	67	25.4	113	244	17.2	158	59.8	30	315
D. pseudoobscura	244	45	56	37.5	109	263	14.4	168	63.7	20	285
Total m		57	97	15.0		293	15.6	163	53.0		
s.e.		4.3	7.8	2.3		55.6	2.4	9.6	7.0		

% = percentage of sperm of the short class; *limit* = threshold value chosen between short and long classes. Other abbreviations as in Table I and II. Data are given in 10^{-3} millimeters.

DISCUSSION AND CONCLUSION

The great length of the sperm of numerous drosophilid species raises some technical problems concerning length determination: very elongated flagella are easily broken during dissection and, taking into account incomplete cells, would both decrease the calculated mean and increase the variance.

For that reason, measurement of mature cysts, assumed to give more reliable data, was preferred in our study for species with longer sperm, *e.g.* in *D. melanogaster*. This method in addition to the use of saline solution instead of fixatives, probably explains the discrepancies between our data and some of those

previously published (Table IV). For extreme lengths of over one centimeter, found for example in *D. littoralis* and *D. hydei*, even the cysts are often broken so that it is very difficult to evaluate intraspecific variability. However, it seems reasonable to conclude that shorter values correspond to incomplete cysts and to consider only the longer measurements as typical of the species.

In contrast, there are no technical difficulties in having complete short sperm which do not break easily. Therefore, the heteromorphism of the distributions in the *D. obscura* group species, which has already been observed by previous investigators (Yanders & Perras, 1960; Beatty & Sidhu, 1970; Policansky, 1970; Beatty & Burgoyne, 1971; Sanger & Miller, 1973; Kurokawa *et al.*, 1974) cannot be accounted for by any technical bias.

Table IV. Synopsis of all sperm length values in millimeters published for D. melanogaster (cysts in Joly, 1987; sperm in all other references).

Authors	*Date*	*Strain*	*Method*	*Length*	*Nb*
Cooper	1950			1.760	5
Yanders & Perras	1960	Oregon-R	acetic formalin	1.751	13
Hess & Meyer	1963	XO		1.100	
		XY		1.750	
		XYY		3.700	
Beatty & Sidhu	1970	Oregon-K	aceto-orceine	1.840	1
Beatty & Burgoyne	1971	Oregon-K	giemsa; aceto-orceine	1.720	49
Gould-Somerot *et al.*	1974	XY Canton-S	saline solution	1.920	6
		XYY	with	1.860	5
		XYT (Y; 3)	9% fetal	1.850	5
		XYY	calf serum	1.690	7
Joly	1987	Ivory Coast	ringer solution	1.846	50
		South Africa	ringer solution	1.933	50
		France	ringer solution	1.915	50

The occurrence of very long male gametes in numerous *Drosophila* species raises several evolutionary questions, to be discussed below. The first concerns the ancestral or primitive state of sperm length. According to theories of modern cladistic systematics, this may be inferred by considering taxonomic outgroups. There are very few cases of animals with such relatively giant sperm. Among these are featherwing beetles (Coleoptera, Ptiliidae) (Dybas & Dybas, 1981; Taylor, 1982) or some ostracods (Bauer, 1940) where sperm may be several times the male length (see review in Sivinski, 1984; Jamieson, 1987b). Nevertheless, species with sperm of inordinate length are still more common in fruit flies. A reasonable proposal is therefore that short sperm are primitive while long sperm are derived (Hihara & Kurokawa, 1987). However, the situation is less clear at a lower level; in the *D. melanogaster* species complex, for instance (Table I), sperm length distributions appear to be divergent in most closely related species *(e.g. D. simulans cf. D. sechellia),* but convergent in less closely related species *(e.g. D. sechellia cf. D.*

melanogaster). Here if phylogeny is considered (Cariou, 1987) we must conclude that elongation occurred independently during the speciation process. A similar reasoning could be applied in comparing other taxa in which there are species with either short or long sperm. Unfortunately, knowledge of *Drosophila* phylogenies does not presently allow such comparison. Whatever the conclusions might be, it remains clear that evolution and speciation in the family Drosophilidae is characterized by a general tendency towards increasing sperm length, as already assumed by Hihara & Kurokawa (1987).

This overall evolutionary tendency further suggests that size variation is not random but has been subject to natural selection (Joly, 1989). The most important questions that then arise are: how and why did sperm elongation evolve? Some insights may be gained by considering the heteromorphism of the *D. obscura* group species.

Clearly, heteromorphism, which is typical of the whole group, is genetically determined (Beatty & Sidhu, 1970). Moreover, this does not correspond to a genetic polymorphism at the diploid level, since any single male produces heteromorphic sperm. Nor is it a case of gametic polymorphism since all the sperm cells, included in the same cysts, and which could be genetically different, exhibit the same length. Heteromorphism seems to be more a case of polyphenotypism which is determined by some unknown physiological mechanisms at the cyst level. A reasonable interpretation is that heteromorphism is an evolutionary stable strategy (ESS) (Maynard Smith, 1974), each sperm class having some adaptive advantage. For instance, the short and long sperm may have differential capacities both to reach the storage organs (preemption capacity) and to resist the second male paragonial substances (antipreemption capacity) when a female remates. A precise formulation of such a hypothesis, which requires comparison of the evolution of both sperm length and mating systems in different species, is proposed in a forthcoming paper.

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