*Present address: Laboratoire communication chimique, LNB-UPR 27-CNRS, 31 chemin J. Aiguier, F-13402 Marseille cedex 9.

**Author for correspondence.

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Can we predict the mating pattern of *Drosophila* females from the sperm length distribution in males?

C. Bressac, D. Joly, J. Devaux and D. Lachaise

Laboratoire de Biologie et Génétique Evolutives, CNRS, F-91198 Gif-sur-Yvette Cedex (France)

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Summary. In order to test the validity of the prediction of the mating pattern of females from the sperm length distribution in males, three species of *Drosophila* were analysed. Males in the three species are equally polygynous but females differ in the level of polyandry. A 'low recurrence polyandry' is observed in the sperm dimorphic species *D. affinis* while a 'high recurrence polyandry' is observed in the sperm monomorphic species *D. latifasciaeformis* and *D. littoralis*. These results are consistent with the hypothesis proposed previously that sperm dimorphism in males can only be maintained by a selective alternative in females (i.e. facultative female polygamy), whereas a stricter mating system (e.g. 'obligatory' polyandry) should only result in sperm monomorphism irrespective of the absolute value of sperm length.

Key words. *Drosophila*; repeat matings; polyandrous pattern diversity; sperm length.

Sperm length varies considerably from one species of *Drosophila* to another[1-3]. Moreover, some species, namely *D. teissieri* of the *melanogaster* species subgroup[4] and all species of the *obscura* group[2,3,5-8], show a striking within-ejaculate sperm length dimorphism.

In order to explain this phenomenon in *D. teissieri* a new hypothesis has been proposed elsewhere, relating sperm length distribution and the female mating system prevailing in a population[4]. It is assumed that sperm dimorphism can only be maintained by disruptive selection where each sperm length class is favoured alternatively. This alternative selective context may be facultative female polygamy. As a consequence, the prediction can be made that sperm monomorphic species will be species where the mating system of females is obligatory (i.e., strict monoandry or strict polyandry).

In the present work we have sought to test further the generality of this hypothesis: is there a close relationship between the distributional pattern of sperm length and the mating system of females in other *Drosophila* species? In other words, can we predict the predominant mating system from the sperm length pattern? For that purpose we have studied the mating system of both sexes (i.e. monogyny versus polygyny in males and monoandry versus polyandry in females) in three species characterized by markedly different sperm length patterns: *Drosophila latifasciaeformis* (*Scaptodrosophila* subgenus) with a unimodal distribution of short sperm (153 ± 1 µm), *D. littoralis* (*Drosophila* subgenus) with a unimodal distribution of giant sperm (maximum measured 19×10^3 µm), and *D. affinis* (*Sophophora* subgenus) with a bimodal distribution of short sperm (112 ± 8 µm and 424 ± 91 µm).

If the 'predominant mating system versus specific sperm length distribution' hypothesis is valid, we should expect to find a basic difference in the mating system of females in the sperm dimorphic *D. affinis* on the one hand and in the sperm monomorphic *D. latifasciaeformis* and *D. littoralis* on the other hand.

The strains used for the three species were founded by more than ten wild-caught inseminated females: *D. latifasciaeformis* (subcosmopolitan species of presumed Afrotropical origin), originated from Marie-galante, Guadaloupe, 1980; *D. littoralis* (Palearctic species) from the Paris area, 1980, and *D. affinis* (Nearctic species) from Atlanta, Georgia, 1987. All had been bred since the first generation on standard cornflour medium at 20 °C. For each series of experiments virgin individuals were isolated within a few minutes of eclosion. The age of sexual maturity was determined for each species and sex by confining repeatedly one female and one male of similar and ascending age, and combining partners of unequal ages. Sexual maturity was assessed as the age when mating occurred in at least 70% ($n \geq 54$) of the tests. In a new series of experiments, virgin males and females were individually aged to maturity in tubes containing standard cornflour medium supplemented with dead yeast. The mating system was studied by determining both the number of times and the periodicity with which each sex was able to remate consecutively with a new virgin partner at the age of maturity in a daily 8-h period (i.e. between 09.00 and 17.00 h).

The copulation duration and between-copulation latencies were recorded. Each test was repeated at least 39 times. Estimates of fecundity of females inseminated by one, two or three males in a 8-h period were obtained by counting the number of eggs produced at 20–23 °C by individually isolated females all through their reproductive life in oviposition boxes, the cornflour-yeast medium of which was changed daily.

Data summarized in table 1 show that the age at maturity in the three species was the same in both sexes, in contrast with what is observed in other *Drosophila* species like *D. hydei*[10]. It is 3, 4 and 5 days in *D. latifasciaeformis*, *D. affinis* and *D. littoralis* respectively.

Tables 1 and 2 show that males in the three species were consistently polygynous and could mate repeatedly with new virgin females: *D. latifasciaeformis* males, up to 8 times, *D. affinis* males up to 9 times, and *D. littoralis* males up to 11 times in an 8-h period (the mean number being statistically constant around 3–4 times for the three species). Evidence was provided by a *D. littoralis* male that had mated with 11 females that the size of the progeny sired by males, although it varied, did not decrease dramatically with the number of matings. The number of eggs produced over a 30-day period being, for example, 404, 681, 802 and 555 for the 1st, 4th, 7th and 10th mating respectively (the 10th mating still resulted in more offspring than the 1st). In these three *Drosophila* species most eggs laid were fertilized (hatching rate was around 0.98). Therefore, productivity gives a valuation of the amount of sperm used and hence a minimal estimate of sperm transferred[9]. This suggests that each copulation, when successful, resulted in effective sperm transfer and fertilization.

In *D. latifasciaeformis* and *D. littoralis* the duration of copulation did not change significantly (a few exceptions are observed with *D. latifasciaeformis* males; the tests between any of the 3rd, 4th or 5th matings with any of the others being not significant, $p < 0.02$). In contrast, in *D. affinis* the copulation duration decreased from the first to the second mating in both sexes. Subsequently, in females it remained constant between the second and fourth matings (mean pairwise comparisons $p < 0.05$) while in males it increased gradually until the 9th mating (mean pairwise comparisons $p < 0.05$ between the 2nd and 6th, 3rd and 7th, 4th and 8th matings). Nevertheless, on the whole the copulation time appeared to be relatively stable within each species (or line[11]) studied, being less than 2 min (100.5 ± 6.2 s, $n = 202$) in *D. affinis* and about 5 min in both *D. latifasciaeformis* (296.8 ± 9.6 s, $n = 204$) and *D. littoralis* (285.5 ± 9.0 s, $n = 157$).

The figure shows that in general (i.e. up to the 5th consecutive mating in an 8-h period) the pre-mating or between-mating latencies in males were shorter in the sperm-dimorphic species (*D. affinis*) than in the two sperm-monomorphic species (*D. littoralis* and *D. latifasciaeformis*). Moreover, up to the 5th mating, the more the number of repeat matings increased, the shorter latency was in *D. affinis*, the longer in *D. latifasciaeformis*.

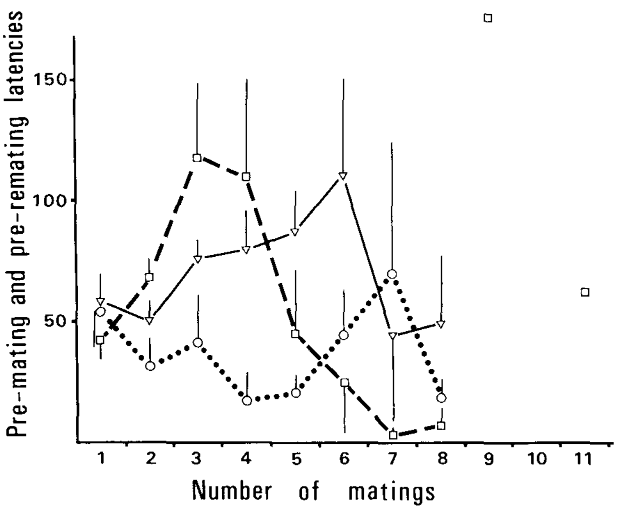
Whatever the differences observed in the polygynous patterns of males may be, the major result concerns differences in the polyandrous patterns of females (table 1). It

Table 1. The mating systems in three unrelated *Drosophila* species characterized by strongly different sperm patterns. Sexual maturity in both sexes, mean number of repeat matings and between-mating latencies in female are given. The 'low recurrence polyandry' observed in *D. affinis* contrasts with the 'high recurrence polyandry' observed in both *D. latifasciaeformis* and *D. littoralis*.

	Sexual maturity in days		Number of pairs successful cf. tested	Mean number of matings ♂ M ± SE	Number of pairs for repeat mating	Mean number of matings ♀ M ± SE	Mean between-mating latencies ♀ (in h)				Duration of the test in h
	♂	♀					1st-2nd M ± SE	Number of pairs remating cf. initial number	2nd-3rd M ± SE	Number of pairs remating cf. initial number	
D. latifasciaeformis	3	3	52/61	3.73 ± 0.26	47	1.30 ± 0.07	2.6 ± 0.4	13/47	0.23	1/47	8
D. littoralis	5	5	53/65	2.85 ± 0.34	39	1.74 ± 0.13	3.7 ± 0.3	20/38	2.6 ± 0.2	8/38	8
D. affinis	4	4	45/54	3.77 ± 0.36	39	2.13 ± 0.16	177.3 ± 27.8	17/23	147.6 ± 26.5	9/23	15 × 8

Table 2. Variation of the copulation duration according to the number of repeat matings in three *Drosophila* species. Mean duration (in s) and standard error are given for each category of mating or remating of either sex (the number of pairs measured are indicated in brackets). A new virgin partner was offered as soon as the previous mating was terminated. The starting males and females of the three species (including *D. affinis* males) were tested over a 8-h period except *D. affinis* females (*) for which the experiments extended over a 15-day period (i.e. including 15 8-h periods for one female). See statistics in text.

Number of repeat matings	Sex	*D. affinis*	*D. latifasciaeformis*	*D. littoralis*
1		116.5 ± 15.7(64)	254.2 ± 19.2(67)	265.0 ± 12.4(57)
2	♂	79.8 ± 9.6(33)	274.8 ± 17.5(42)	267.3 ± 8.7(27)
	♀	*77.7 ± 4.8(17)	266.5 ± 24.0(13)	288.5 ± 14.9(20)
3	♂	82.8 ± 13.4(23)	335.5 ± 22.8(32)	295.4 ± 17.1(18)
	♀	*72.1 ± 6.5(9)	506.0(1)	298.5 ± 17.1(8)
4	♂	88.4 ± 12.3(20)	354.1 ± 22.7(23)	265.5 ± 16.3(11)
	♀	*73.3 ± 13.0(3)	–	–
5	♂	102.3 ± 21.3(12)	391.2 ± 40.8(15)	285.3 ± 22.5(6)
	♀	–	–	–
6	♂	142.1 ± 32.8(11)	286.5 ± 32.9(6)	316.3 ± 42.7(3)
	♀	–	–	–
7	♂	149.0 ± 35.6(6)	319.7 ± 55.0(3)	317.0 ± 81.0(2)
	♀	–	–	–
8	♂	150.3 ± 24.7(3)	290.5 ± 4.5(2)	285.5 ± 47.5(2)
	♀	–	–	–
9	♂	205.0(1)	–	305.0(1)
	♀	–	–	–
10	♂	–	–	265.0(1)
	♀	–	–	–
11	♂	–	–	255.0(1)
	♀	–	–	–



Mean pre-mating and pre-remating latencies in min (error bars indicate 1 SE) plotted against the number of repeat matings in males of three *Drosophila* species: *D. latifasciaeformis* (continuous line), *D. littoralis* (dashed line) and *D. affinis* (dotted line). The two isolated squares correspond to the 10th and 11th mating of one *D. littoralis* male. Males were offered new virgin females after each mating all along a 8-h period.

is worth stressing that although the three species are globally polyandrous, two strongly different mating patterns in females can be recognized. We call them 'high recurrence polyandry' in *D. littoralis* and *D. latifasciaeformis* (rematings are closely spaced, occurring every 2.30–3.30 h) versus 'low recurrence polyandry' in *D. affinis* (two rematings occurred every 7 days). Females of both *D. latifasciaeformis* and *D. littoralis* can mate 2 and even 3 times in an 8-h period whereas *D. affinis* requires a 15-day period (confinement of the sexes together lasting 8 h each day), to get a comparable number of repeat matings. In a single 8-h period *D. affinis* is strictly monoandrous. The mean between latencies in females tends to decrease slightly in the three species as the frequency of remating increases, although not significantly in *D. affinis* ($p < 0.05$).

There is effective polyandry if, and only if, remating occurs before the stored sperm supply is significantly tapped. In this case, its main consequence is to superimpose between-ejaculate competition on the within-ejaculate sperm competition preexisting in the storage organs of females. Assuming that these latter remain unsaturated (i.e. incompletely filled) after the first insemination, polyandry may result in increased productivity. Productivity corresponds to the mean number of fertilized eggs oviposited by females all through their reproductive life. Clearly, the mean output per female in both *D. latifasciaeformis* and *D. littoralis* increases gradually with the number of repeat matings (mean comparisons $p < 0.05$, table 3). Considering that both *D. latifasciaeformis* and *D. littoralis* fail to lay eggs between the two (or three) consecutive matings occurring in an 8-h period, this indicates that the storage organs remain unsaturated after the first mating in the former species and the two first matings in the latter.

In *D. affinis*, fecundity in repeatedly-mated females was followed only during 15 days after the first mating (i.e.

Table 3. Relationship between productivity and remating in three species of *Drosophila* over an 8-h period. Productivity corresponds to the mean number of eggs ± standard error (number of females tested in brackets) oviposited by females all along their reproductive life or over a 15-day period post first mating.

Over an 8-h period	Number of females	Mean reproductive life ± SE of females (in days)	Mean productivity per female all along their life			
			1 mating	2 matings	3 matings	
D. latifasciaeformis	8	60.2 ± 2.5	754 ± 168 (4)	1028 ± 39 (4)	–	
D. littoralis	11	59.1 ± 2.9	939 ± 206 (6)	1029 ± 18 (2)	1667 ± 321 (3)	
D. affinis	10	42.4 ± 2.6	579 ± 108 (10)	–	–	
Over a 15-day period			Over a 15-day period after the 1st mating			
			1 mating	2 matings	3 matings	4 matings
D. affinis			217 ± 14 (6)	242 ± 18 (6)	210 ± 14 (6)	253 ± 2 (2)

nearly 35% of the reproductive life). Interestingly, the mean productivity per female remained constant after one, two, three or four matings (mean comparisons $p < 0.05$, table 3). However, there is evidence that eggs were laid between two consecutive matings (e.g. 1st–2nd: 108.5 ± 29, n = 14, 2nd–3rd: 95.1 ± 14.8, n = 8; 3rd–4th: 62.5 ± 32.2, n = 2) even though the stored sperm supply was only reduced by half. From these considerations it appears that repeat matings in *D. affinis* result in equal gain and cost. Remating results in a cost because, in our experiment, those females that mate four times in a 15-day period interrupted oviposition during about 8 days (i.e. 4 × 2 days post-remating). This cost is approximately balanced by the female storage-organ replenishment (gain) following each reinsemination. The higher the level of stored sperm supply, the longer the latencies are assumed to be[12].

In conclusion, a major datum to emphasize is that there is a striking dissimilarity in the polyandrous patterns in the three species, which contrasts with the between-species similarity in the polygynous patterns. Therefore, there is predominance of female behaviour over that of males in determining the species-specific patterns of the mating systems. Considering that males in the three species are highly polygynous, if females are also highly polyandrous, as is observed in *D. latifasciaeformis* and *D. littoralis*, the probability that males will mate with previously inseminated females is great. In other words, both species can be considered as being strictly polyandrous. This result is consistent with the expectation stated above about the basis of monomorphic sperm length patterns, even though the absolute species-specific sperm length values differ strongly. To explain this difference, it can simply be assumed that the process of sperm elongation has undergone a runaway evolution in *D. littoralis* but not in *D. latifasciaeformis*. In this respect, it is worth noting that in a sperm dimorphic species like *D. obscura* the mean values of the short and long sperm length classes are 76 and 139 μm respectively[3]. If a unidirectional selection were to occur in this species, favouring longer sperm, it would result in a monomorphic pattern with a sperm length still lower than that observed in *D. latifasciaeformis*. Interestingly, the association of obligatory polyandry[10] with giant sperm monomorphism[13] is also known in *D. hydei*, and that of obligatory monoandry[14] with mid-length sperm monomorphism[15] in *D. nasuta*. In contrast, the mating pattern observed in the sperm dimorphic species *D. affinis*, consisting of 'low recurrence polyandry', allows either monoandry or polyandry. This contingency leaves a clear field for selective alternatives, a condition which was precisely required in the starting hypothesis[4] to account for the maintenance of sperm dimorphism.

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