propane ring might have an important role in stabilizing the three-dimensional structure of the molecule, and thus favor its binding to a target molecule and/or generating a reactive species[18]. Creatine itself could not be detected in *Gonyaulax* extracts (for method of detection see[19]); however, judged by its τ-shortening capacities (fig. 3), creatine might be a potent and non-metabolizable analog of gonyauline.

Since gonyauline is related to methionine, a possible mechanism for the τ-effect might involve the regulation of proteins and/or nucleic acids by methylation[20] and, because of the spectral dependence of the effects, these regulations might be found in cellular pathways of light transduction. However, it is too early to speculate whether gonyauline is produced specifically for the purpose of modulating the frequency of the circadian clock or whether it also serves other functions; further studies are required to elucidate the biochemical mechanisms underlying the effects of both gonyauline and creatine on the circadian period.

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The postpharyngeal glands and the cuticle of Formicidae contain the same characteristic hydrocarbons

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Summary. Comparison of the contents of the postpharyngeal gland and cuticular hydrocarbons of five species of ant have shown them to contain the same compounds and to be characteristic of the species. For four species (*Formica selysi, Camponotus lateralis, Camponotus vagus* and *Manica rubida*), quantitative similarity was very close, but more divergent in the fifth (*Myrmica rubra*). Glands and cuticle of *M. rubra* queens were shown to be closely similar to those of workers, except the glands of queens are larger, but the cuticle of larvae was different from that of adult cuticle and postpharyngeal glands.
Key words. Formicidae; ants; postpharyngeal gland; cuticle; hydrocarbons; larvae; queens; workers.

The postpharyngeal gland, found in all castes of all species of the Formicidae, occupies an important part of the cephalic capsule. It is usually described as having finger-like projections and is filled with a clear or pale yellow fluid. The structure and contents of this unique organ have been little studied, and its function remains unknown today. Peregrine et al.[1,2] and Delage-Darchen[3] have described the gland and considered various suggestions for its function. Partly because it is connected directly to the pharynx, but also from the results of some labelling experiments, most authors attribute to it an alimentary role. The known chemistry of the gland was recently reviewed[4].

Brian and Blum[5] demonstrated the importance of the head of *Myrmica rubra* queens for the control of growth of their larvae and suggested that fatty acids, possibly

from the postpharyngeal or mandibular glands were the active substances. Markin[6] similarly described the importance of the postpharyngeal glands of *Iridomyrmex* queens for the growth of larvae.

The work of Vinson et al.[7], Thompson et al.[8], and Vander Meer et al.[9], on glands of queens of *Solenopsis invicta*, demonstrated that a number of hydrocarbons are present in the gland of this species. Thompson et al.[8] noted in passing that the major hydrocarbons of the queen's postpharyngeal gland were also present in the cuticle, but did not investigate this further. Brill and Bertsch[10] in an investigation of sampling methods for cuticular hydrocarbons, also noted that the postpharyngeal gland of *S. richteri* contained hydrocarbons identical to those in the cuticle. Attygalle et al.[11] showed that the very large gland of *Solenopsis geminata* workers was filled with a mixture of liquid hydrocarbons with (Z)-9-tricosene the major component.

We have recently developed a method for direct chromatographic sampling of cuticular wax of insects, without the use of solvents[12] to complement the extensive studies of one of us on the chemical composition of the cuticular wax of ants and termites and their role in species and colony recognition[13]. Using also our technique for chromatographic examination of single, dissected glands, we have been able to compare cuticle wax and postpharyngeal glands without fear of contamination of one with the other. We now wish to draw attention to the fact that the mixture of oily hydrocarbons of the postpharyngeal gland closely resembles the mixture of hydrocarbons on the cuticle of that species.

Materials and methods

Colonies of *Myrmica rubra* were collected in Staffordshire, *Manica rubida* and *Formica selysi* were collected in the French Alps and *Camponotus lateralis* and *Camponotus vagus* in southern France. All species were maintained live in artificial nests in the laboratory and prepared for dissection immediately before chromatography, by cooling in the refrigerator. Dissection was made in distilled water under a binocular microscope with fine forceps. The gland or cuticle was placed on a tiny fragment of glass and dried by touching with a fragment of tissue paper. The sample was then dropped into a glass capillary, sealed at one end and the other end sealed in a flame[14]. The contents of the glass capillary were then chromatographed by the solvent-less technique of Morgan and Wadhams[15] (see also Bagnères and Morgan[12]).

Gas chromatography-mass spectrometry was performed on a Hewlett Packard 5890 Gas Chromatograph and 5970B Mass Selective Detector (quadrupole mass spectrometer using 70eV electron impact ionization). The whole system was controlled by the Hewlett Packard Series 300 computer with HP 59970C Chemstation.

Chromatography was carried out on an immobilized polydimethylsiloxane phase (equivalent to OV-1) in a fused silica capillary column (12 m × 0.2 mm, 0.33 μm film thickness, SAC Chromatography, Letchworth, UK), linked directly to the mass spectrometer source by a 10 m length of 0.2 mm i.d. deactivated fused silica capillary (SAC Chromatography). Helium was used as carrier gas at 1 ml min^{-1}. The sample was heated in the injector to 250 °C for 5 min before crushing. The oven was programmed from 150 °C at 3° min^{-1} to 275 °C and then held isothermal. The split vent was closed before crushing the sample and reopened 30 s later.

As retention time and external quantitation standards, ng samples of C_{18} to C_{30} saturated hydrocarbons and pure Z-9-tricosene in hexane solutions were chromatographed under the same conditions. Identifications were based upon mass spectra. The position of methyl-branches were revealed by relatively intense ions corresponding to preferred cleavage at the branching points[16]. Where double bond positions are given, these were obtained from the mass spectra of the products of methylthiolation of the alkenes with dimethyl disulphide[17,18]. These determinations were carried out for the cuticular hydrocarbons of each of the species, and are described in greater detail in the references cited for these species.

Results

The cuticular hydrocarbons of *Formica selysi* and *Manica rubida* were studied first since these have been extensively examined and the hydrocarbons identified[19]. The pattern of substances obtained (figs 1a, 2a) corresponded completely to that formerly obtained by conventional

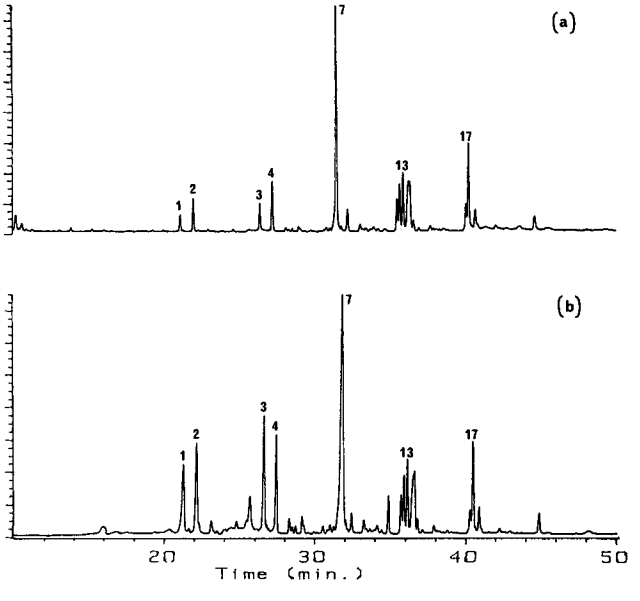

Figure 1. Gas chromatograms of (a) piece of cuticle from the abdomen of a single worker of *Formica selysi* and (b) the contents of two post-pharyngeal glands. For identification of numbered peaks, see table. The average quantity was 2.3 μg hydrocarbons per gland. Peak 7, 9-heptacosene represents about 25% of the total and 0.2 μg.

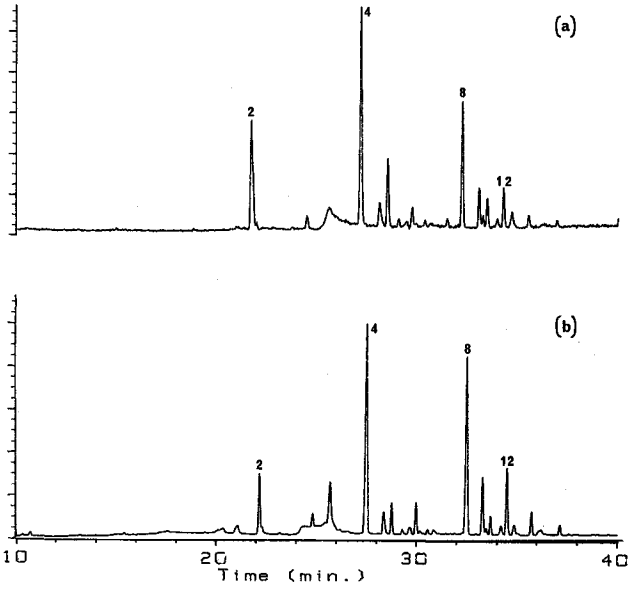

Figure 2. Gas chromatograms of (a) cuticle from two antennae of a single worker of *Manica rubida* and (b) contents of a single worker's postpharyngeal gland from the same species. Numbered peaks are identified in the table. The two antennae contained 244 µg of hydrocarbons and the postpharyngeal gland 3 µg. Peak 4 represents 25% of total on both, 60 ng on the antennae and 680 ng in the gland.

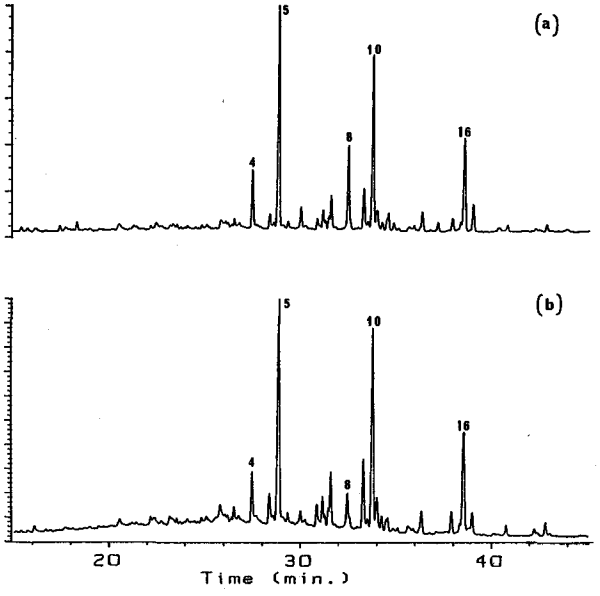

Figure 3. Gas chromatograms of (a) cuticle from the abdomen of a worker of *Camponotus lateralis* and (b) contents of the postpharyngeal gland of a worker from the same colony. Numbered peaks are identified in the table. The average quantity in the gland was 3.5 µg with 5-methylpentacosane (peak 5) representing about 20% of the total or 0.7 µg.

solvent extraction methods for both species. The composition of the contents of the postpharyngeal gland of both species (figs 1b, 2b) matched completely the substances and closely resembled their proportions in the cuticle of that species.

For *F. selysi* (Formicinae), 32 compounds were identified on the cuticle [19] and in the postpharyngeal gland, ranging in molecular size from 23 to 33 carbon atoms. 9-Heptacosene (**7** in fig. 1 and table) is the major component, followed by pentacosane (**4**) and 9-nonacosene in both gland and cuticle. Some 26 hydrocarbons were identified on the cuticle of *Manica rubida* (Myrmicinae) between C_{21} and C_{29}, with tricosane (**2**), pentacosane (**4**) and heptacosane (**8**) the major components, with the same pattern in the postpharyngeal gland (fig. 2 and table). The proportions varied a little from one individual to another, as we have found in examining the Dufour gland contents of numerous species [20]. While proportions vary a little with individuals, they remain within the range of values characteristic of species.

Some of the substances identified in the postpharyngeal glands and the cuticle of the species of ant shown in figures 1–6

Number in figure	Compound	Species					
		F. selysi	*C. lateralis*	*M. rubida*	*M. rubra*		
					Queen	Worker	Larva
1	9-Tricosene	cP*	–	–	–	–	–
2	Tricosane	cP	–	cP	cp	cp	cp
3	9-Pentacosene	cP	–	–	–	–	–
4	Pentacosane	CP	cp	CP	Cp	CP	C
5	5-Methylpentacosane	–	CP	Cp	cP	cP	–
6	5,11-Dimethylpentacosane	–	–	–	cP	cP	c
7	9-Heptacosene	CP	–	–	–	–	–
8	Heptacosane	cp	cp	CP	c–	c–	C
9	11- and 13-Methylheptacosane	cp	cp	cP	CP	CP	c
10	5-Methylheptacosane	–	CP	cp	Cp	Cp	c
11	5,11-Dimethylheptacosane	–	–	–	CP	CP	c
12	5,17-Dimethylheptacosane	–	–	Cp	–	–	–
13	9,23-Nonacosadiene	CP	–	–	–	–	–
14	Nonacosane	–	–	–	–	c–	–
15	11- and 13-Methylnonacosane	–	cp	–	cP	CP	c
16	11,14-Dimethylnonacosane	–	CP	–	–	–	–
17	9,21- and 9,23-Hentriacontadiene	CP	–	–	–	–	–

*c = minor cuticle component; C, major component. p = minor postpharyngeal gland component; P, major component.
A blank does not mean the compound is entirely absent from the mixture, it may be present as a trace.

The cuticular hydrocarbons of *Camponotus lateralis* (Formicinae) have also been identified as part of a study on intraspecific recognition[21]. We therefore examined this species as representative of a rather different group of formicines. In all, 31 saturated alkanes from C_{25} to C_{32} have been identified in its cuticular wax, with pentacosane, 5-methylpentacosane, 6-methylhexacosane, heptacosane, a mixture of 11- and 13-methylheptacosane and 11,14-dimethylnonacosane as the major substances. Different colonies of *C. lateralis* contain the same cuticular hydrocarbons but there appears to be considerable variation in their relative proportions. For a particular colony, we have found very close agreement in the pattern in both cuticle and gland as illustrated in figure 3. They may be compared with the patterns given by Bonavita-Cougourdan et al.[21]. We have also compared the cuticle and postpharyngeal glands of *Camponotus vagus* in this study, and have found them to give the same chromatographic pattern, with mixtures of 15-, 13- and 11-methylhentriacontane, 15-, 13- and 11-methyltritriacontane, and 2,19-, 3,21- and 4,23-dimethyltritriacontane, identical to that already determined for the cuticle[22] by solvent extraction.

Myrmica rubra (Myrmicinae) was available as a large, vigorous colony with queens and larvae, established in the laboratory for four years. There was surprisingly good agreement between worker cuticle from our laboratory colony from Staffordshire (fig. 4a) and those collected in France[23]. There were obvious differences between worker cuticle and postpharyngeal glands of the English colony of *M. rubra* (fig. 4b). Alkenes and normal alkanes, in particular pentacosane (**4**), heptacosane (**8**) and nonacosane (**14**) are relatively more abundant in the cuticle, with branched alkanes, principally a mixture of 11- and 13-methylalkanes more abundant in the gland. The chromatograms obtained for postpharyngeal glands of queens were very close in appearance to those of workers from the same colony (fig. 5). The 33 identified peaks were the same as those shown in figure 3. There was no indication of large amounts of the free fatty acids found in queens' heads by Brian and Blum[5]. The appearance of larval cuticle (fig. 6) was closer to that of queens than to that of workers. The substances were the same but there was much more pentacosane in larval cuticle.

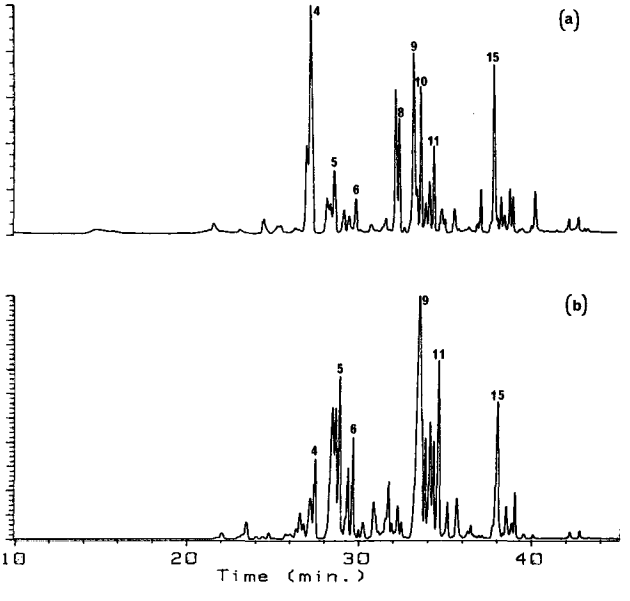

Figure 4. Gas chromatograms of (a) a piece of cuticle from the abdomen of a worker of *Myrmica rubra* and (b) contents of the postpharyngeal gland. Numbered peaks are identified in the table. The gland illustrated contained 5.8 µg of hydrocarbons with peak 9 representing 22% (1.3 µg) of the total. Individual glands contained from 3 to 6 µg.

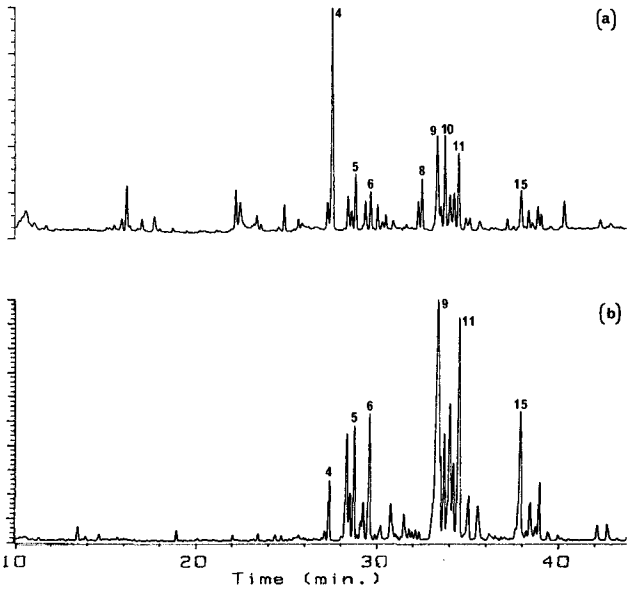

Figure 5. Gas chromatograms of (a) cuticle from the two forelegs of a queen (dealate female) of *Myrmica rubra* and (b) contents of her postpharyngeal gland. Numbered peaks are identified in the table. The two legs contained approximately 2.6 µg of hydrocarbons with peak 4 (pentacosane) representing 420 ng (16%). The gland illustrated contained 2.6 µg of hydrocarbons. The queens' glands were not larger than worker glands.

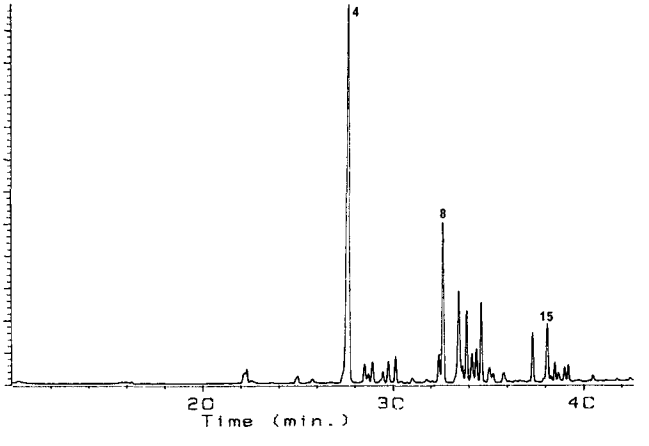

Figure 6. Gas chromatogram of the cuticular hydrocarbons of a single larva of *Myrmica rubra*. The major peak, No. 4 contains 667 ng, 38% of the total. The total quantity of hydrocarbons on the cuticle was about 1.7 µg.

The identification of these substances was made by comparison of their retention times with standard hydrocarbons and their mass spectra with those already published or to be published by Bagnères et al.[19], Vienne et al.[23], Bonavita-Cougourdan et al.[21], and Bonavita et al.[22]. Chromatography of other tissues from the cephalic capsule showed that they did not contain hydrocarbons.

Discussion

Previous investigations on postpharyngeal glands have concentrated chiefly on those of queens[6-9] though we have made a preliminary study of the glands of workers of one species, *Solenopsis geminata*[11]. The similarity of the hydrocarbons we found in *S. geminata* to typical cuticular hydrocarbons and the statement of Thompson et al.[8] that the major substances of *S. invicta* queen postpharyngeal glands were the same as the major hydrocarbons of their cuticle caused us to look more closely at this possible relationship. In several species of ant, both formicine and myrmicine, we find the contents of the postpharyngeal gland match the pattern of cuticular hydrocarbons. Only in one of the five species examined, *Myrmica rubra*, was there an obvious difference in the proportion of substances present between gland and cuticle. In *M. rubra*, the contents of glands of queens and workers were the same, but the cuticle of queens, workers and larvae differed in the proportions of the substances. Some indication of the quantity of substances in the glands, and on parts of the body cuticle are given in the figure captions. The clear translucent appearance of the gland showed it to have a homogeneous oily content, unmixed with any aqueous phase.

It is noteworthy that although the normal hydrocarbons in the range examined here (C_{20} to C_{32}) are all high-melting solids (eicosane m.p. 38 °C; triacontane m.p. 66 °C) the mixture in the gland is always liquid at room temperature. This is because of the presence of the alkenes, which have much lower melting points (for example, tricosane has m.p. 48 °C, (Z)-9-tricosene is a liquid at room temp.), and the methylbranched alkanes. There is little information available on how much the introduction of a methyl branch lowers the melting point of a hydrocarbon, but it is apparent that the branched hydrocarbons present, even with large amounts of linear alkanes in some of the mixtures, are sufficient to maintain the postpharyngeal gland contents as liquids. It is noteworthy that while *F. selysi* contained alkenes in both gland and cuticle, *C. lateralis* contains none. There is a higher proportion of the linear hydrocarbons on the cuticle, so that there the mixture will be closer to a soft wax, but there is no direct evidence that the cuticle is an oil or wax.

The demonstration that larvae have different cuticular hydrocarbons from the adult workers in *M. rubra* is also interesting. The huge peak for solid pentacosane of these larvae probably means that they are indeed covered with a cuticular wax. It has been pointed out that in the carpet beetle *Attagenus megatoma*, there is a shift from an abrasion-resistant hard wax in the larvae to a softer cuticular wax in the adults[24].

The idea that the postpharyngeal gland has an alimentary role is not attractive biochemically. Hydrocarbons are not easily digested by insects or most micro-organisms, their use as protection for the cuticle bears that out. Our results show there is a very close relationship between gland and cuticle. What that relationship is, remains unclear. One possibility is that the gland may serve a purely mechanical purpose in providing lubrication and a softener for the cuticular wax. There is however also a possible social function, if the glandular contents are regurgitated as a colony, species, or caste marker.

Numerous studies have shown that cuticular hydrocarbons provide a sort of 'chemical signature' characteristic of a species, and possibly also of a colony and of a caste[25]. Bonavita-Cougourdan et al.[26] noted the presence of some major cuticular hydrocarbons in the postpharyngeal glands in *Camponotus vagus*. They briefly mention that a hexane extract of postpharyngeal gland applied to homocolonial workers of *C. vagus* aroused no aggressive behaviour from their nestmates, but alien gland extract released a high level of aggressive behaviour[22]. The recent work of Vienne et al.[23] on the influence of the queen on mixed colonies of *Myrmica rubra* and *Manica rubida* suggests that the cuticular hydrocarbons of the mixed colony is influenced by whichever queen is present. Although the groups of workers of the two species are observed to avoid each other within the nest and not to practice mutual grooming[23], we must consider the possibility that the postpharyngeal gland contents of the queen may be passed in some way to all the workers of both species in the nest, altering their cuticular profile. Similarly, the work of Bagnères et al.[19] showed that mixed queenless colonies of *F. selysi* and *M. rubida* acquired different hydrocarbon profiles from unmixed colonies, and the new profiles were more like the species sharing the nest. The absence of queens does not inhibit modification of the hydrocarbon profile. In spite of the lack of observation of mutual grooming, one of us suggests that the workers in these mixed groups may exchange their postpharyngeal gland contents. If these glandular substances are used to reinforce the chemical signature of the mixed colonies, it would help to explain the increased amount of cuticular hydrocarbons on workers of the mixed colony. The present work shows that the function of the postpharyngeal gland must be totally reconsidered.

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

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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 $\pm$ 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 $\pm$ 8 µm and 424 $\pm$ 91 µm).