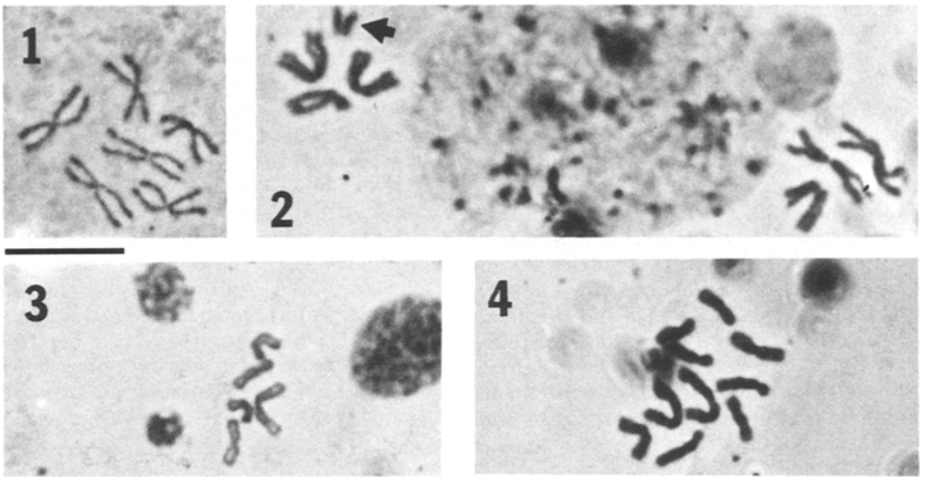

Chromosomes of *Brachymeria* spp. Figure 1. Diploid karyotype of *B. intermedia* from brain cell of early instar larva. Figure 2. Haploid karyotypes of *B. intermedia* showing acentric segment (arrowed) resulting from breakage at the site of secondary constriction and a normal haploid chromosome set (lower right) from spermatocyte. Figure 3. Haploid karyotype of *B. lasus* from spermatocyte. Figure 4. Diploid karyotype of *B. ovata* from brain cell of early instar larva. Scale = 10 μm.

zona. Aceto-orcein squash[5] and air-drying[6] techniques were used in chromosome preparation. Karyotype determinations were based on at least 20 cells with well-spread chromosomes, except in *B. lasus* where only 6 cells from one male were used.

Results and discussion. Four submetacentric (SM) and two metacentric (M) chromosomes were observed in both somatic and germ cells of females in *B. intermedia* (fig. 1). Although a secondary constriction was not clearly demonstrated in photographic preparations, an actual breakage was observed in two cells from testes of two males (fig. 2). However, this breakage was not found in the other four males used in this study. Although the two other species of *Brachymeria* have the same haploid number of five (n = 5), their karyotypes are different. *Brachymeria lasus* has K = 3M+1SM+1A (fig. 3), but *B. ovata* has K = 3SM+2A (fig. 4).

The lowest chromosome number in animals so far found is in the false spider mites[7], with n = 2. Recently, Ferreira et al.[8] also found n = 2 in the *Chalcolepidius* beetle which represents the lowest chromosome number in Coleoptera. The haploid number of five reported here for both *B. lasus* and *B. ovata* is the modal value for the superfamily Chalcidoidea[9]. Goodpasture reported n = 4 in *Monodontomerus obscurus*[10]. Although a haploid number of three has been found in the bee *Hesperandrena duboisi*[11], this report of n = 3 in *B. intermedia* is the first published document of the lowest chromosome number in the order Hymenoptera.

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2 Crozier, R. H., A. Rev. Ent. *22* (1977) 263.
3 Imai, H. T., and Kubota, M., Chromosoma *37* (1972) 193.
4 Burks, B. D., Ent. News *71* (1960) 62.
5 Hung, A. C. F., Imai, H. T., and Kubota, M., A. ent. Soc. Amer. *62* (1977) 455.
6 Imai, H. T., Crozier, R. H., and Taylor, R. W., Chromosoma *59* (1977) 341.
7 Helle, W., Bolland, H. R., and Gutierrez, J., Experientia *28* (1972) 707.
8 Ferreira, A., Cella, D. M., Ramos Tardivo, J., and Virkki, N., Rev. Brasil. Genet. *7* (1984) 231.
9 Goodpasture, C., and Grissell, E. E., Can. J. Genet. Cytol. *17* (1975) 413.
10 Goodpasture, C., Ann. ent. Soc. Amer. *68* (1975) 391.
11 Goodpasture, C., Cytological data and its uses in the classification of the Hymenoptera. Ph. D. thesis, University of California, Davis 1974.

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New contribution to the study of chromosomes of the European Cryptocephalinae (Coleoptera, Chrysomelidae)

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Summary. The chromosomes of three species of *Pachybrachis* and nine of *Cryptocephalus* chrysomelids were analyzed. The male meiotic bivalent formula of *P. azureus* Suffr., *P. catalonicus* Burl. and *P. petitpierrei* Daccordi is $7^{II}+Xy_r$. *Cryptocephalus sexmaculatus* Ol. and *C. vittula* Suffr. have $13^{II}+Xy_p$, *C. bipunctatus* L. $14^{II}+Xy_r$, *C. ochroleucus* Steph. and *C. ocellatus* Drap. $14^{II}+Xy_p$, *C. crassus* 01. $15^{II}+Xy_r$, *C. sulphureus* 01. $15^{II}+Xy_p$, the same number as in *C. fulvus* Goeze with 2n=32 chromosomes, while *C. primarius* Har. has $19^{II}+Xy_p$. The modal chromosome number in *Cryptocephalus* is 2n=30 (about 60% of spp.), and most species are characterized by their small chromosomes. The low variation found in the karyotypes of Cryptocephalinae along with their possible interrelationships with allied chrysomelid subfamilies are also discussed.

Key words. Chromosomes; cytogenetic evolution, Cryptocephalinae; chrysomelid beetles.

Table 1. Chromosomally sampled male European Cryptocephalinae

Species	Source	Indiv. checked
Pachybrachis azureus	La Garriga (Barcelona, Sp.)	2
P. catalonicus	Montesquiu (Barcelona, Sp.)	5
	Gironella (Barcelona, Sp.)	6
	Planoles (Girona, Sp.)	1
P. petitpierrei	La Garriga (Barcelona, Sp.)	8
Cryptocephalus bipunctatus	Arbùcies (Girona, Sp.)	5
	Navars (Girona, Sp.)	6
	Olot (Girona, Sp.)	1
	Vidrà (Girona, Sp.)	3
C. crassus	La Garriga (Barcelona, Sp.)	6
C. fulvus	Vallcebre (Barcelona, Sp.)	2
	Sils (Girona, Sp).	6
C. ochroleucus	La Garriga (Barcelona, Sp.)	5
	Ribes de Freser (Girona, Sp.)	3
C. ocellatus	Montesquiu (Barcelona, Sp.)	6
	Planoles (Girona, Sp.)	3
C. primarius	Collsuspina (Barcelona, Sp.)	2
	Vallcebre (Barcelona, Sp.)	3
C. sexmaculatus	La Garriga (Barcelona, Sp.)	2
	Valls (Tarragona, Sp.)	1
C. sulphureus	La Garriga (Barcelona, Sp.)	5
C. vittula	Valcebre (Barcelona, Sp.)	1
	Queralbs (Girona, Sp.)	4
	Vidrà (Girona, Sp.)	3

After our first paper dealing with the chromosomes of European *Cryptocephalus*[2] we have enlarged the cytogenetically sampled species of this genus with additional analyses, and we have also performed studies on some species of the related genus *Pachybrachis*. These two taxa of Cryptocephalinae are genera very rich in species. One taxon has an almost ubiquitous distribution; the other is found mainly in the holarctic region. So they offer suitable material for karyological analyses in closely related organisms. Eight species of *Cryptocephalus* were previously checked[2] and all of them shared 2n=30 chromosomes and a Xy_r male sex-determining system of the 'rod' type, that is a terminal association between the male sex chromosomes giving a rod-shaped appearence. In spite of the great uniformity of the number of chromosomes in *Cryptocephalus*, since 13 out of the 18 known species had 2n=30, their range was quite large, from 2n=16 to 2n=32 chromosomes. The new findings reported herein provide a deeper understanding on the chromosome sets and the karyological evolution of *Cryptocephalus* and gain some insight into those of *Pachybrachis*.

Materials and methods. The chromosomes of three species of *Pachybrachis* and nine of *Cryptocephalus* have been examined in the present work. These species and their geographic sources are given in table 1. The techniques used were two: the conventional aceto-orcein squash of torn testes and the air-dried preparations of the same tissues, previously fixed in a solution of ethanol-acetic acid (3:1), and finally stained with 4% Giemsa in phosphate buffer (pH 6.8).

Results. The three checked species of *Pachybrachis* shared seven autosomal bivalents and the Xy_r sex-determining system (figs 1–3). The spermatogonial metaphases of *P. azureus* and *P. catalonicus* showed 16 chromosomes, most of medium size, and a minute *y*-chromosome. Both species seem to possess three pairs of metacentric/submetacentric and four of acrocentric autosomes plus a large metacentric X and a very small *y*-chromosome (figs 4 and 5). Thus the number of major chromosomal arms or 'nombre fondamentale' (NF) is 23. The metaphases I obtained in eight species of *Cryptocephalus* supplied the following results: in *C. primarius* 19 rather small autosomal bivalents and the Xy_p sex-determining system (fig. 6), in *C. vittula* 13 medium or small autosomal bivalents and the Xy_p sex-determining system (fig. 7), in *C. sulphureus* and *C. crassus* 15 quite similar autosomal bivalents plus the sex-determining system (figs 8 and 9), in *C. bipunctatus*, *C. ocellatus* and *C. ochroleucus* 15 bivalents including the sex-determining system (figs 10–12), whereas in *C. sexmaculatus* only fourteen bivalents were present among which a large autosomal bivalent and the Xy_p sex-determining system were clearly prominent (fig. 13). The spermatogonial metaphases of *Cryptocephalus* gave in *C. primarius* 2n=40 chromosomes (fig. 14), in *C. vittula* 2n=28 (fig. 15), in *C. crassus* 2n=32 (fig. 16), in *C. ocellatus* and *C. ochroleucus* 2n=30 (figs 17 and 18), in *C. sexmaculatus* 2n=28 (fig. 19), and in *C. fulvus* 2n=32 chromosomes (fig. 20). Most chromosomes of these species were of small size and presumably metacentrics, though the centromere locations are not easily visible in the micrographs. The most remarkable features in the whole karyotypes of *Cryptocephalus* were the large metacentric X-chromosome of *C. primarius* (fig. 14) and an outstanding long pair of autosomes in *C. vittula* and *C. sexmaculatus* (figs 15 and 19).

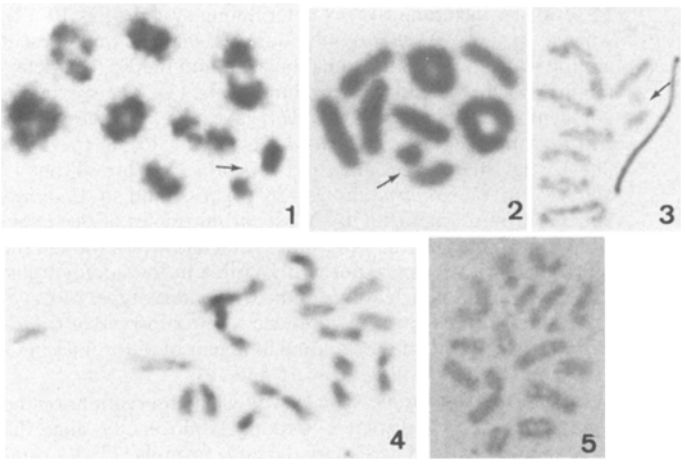
Discussion and conclusions. The karyological observations on the genus *Pachybrachis* display a striking homogeneity since the three species analyzed here share the same formula, $7^{II}+Xy_r$, and rough chromosomal features, despite not being closely related in morphology and intrageneric taxonomy. As far as we know the species of *Pachybrachis* seem to be highly conservative on the karyological grounds since the currently checked species plus three North American ones have the same number of 2n=16 chromosomes. The unique clearly evident difference among the karyotypes of these species of *Pachybrachis* refers to the sex-determining system, one of the species having the 'parachute' system, Xy_p, while all the remaining ones show the 'rod'one, Xy_r (table 2).

The range of chromosome numbers in the species of *Cryptocephalus* is more than twofold, from 2n=16 to 2n=40 chromosomes, but 16 out of the 27 known species have 2n=30 chromo-

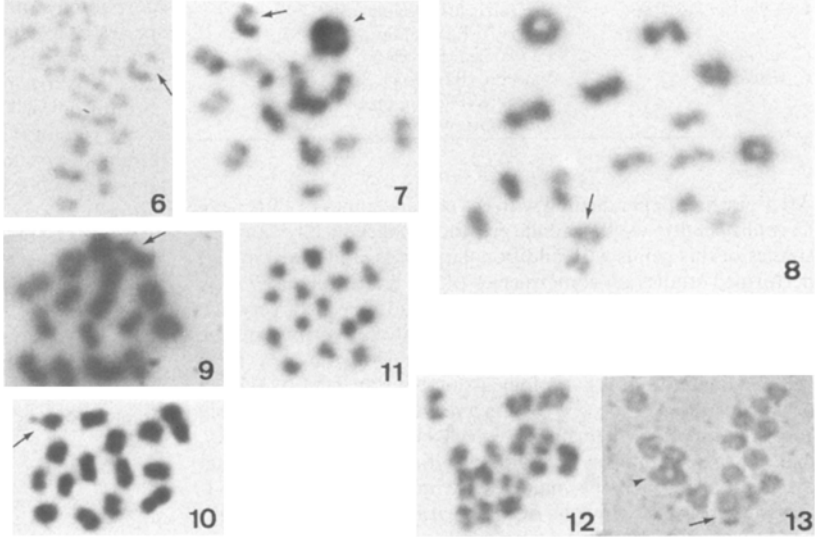
Table 2. Chromosome numbers and male sex-determining systems in Cryptocephalinae

Species	2n	Karyotypic formula	References
Pachybrachis azureus Suffr.	16	$7^{II} + Xy_r$	*
P. bivittatus Say		$7^{II} + Xy_r$	Smith[3]
P. catalonicus Burl.	16	$7^{II} + Xy_r$	*
P. melanostrictus Suffr.		$7^{II} + Xy_r$	Smith[3]
P. peccans Suffr.		$7^{II} + Xy_p$	Smith[3]
P. petitpierrei Daccordi	16	$7^{II} + Xy_r$	*
Cryptocephalus analis Ol.	30	$14^{II} + Xy_p$	Yadav[4]
C. aureolus Suffr.		$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. bipunctatus L.	30	$14^{II} + Xy_r$	*
C. capucinus Suffr.	30	$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. crassus Ol.	32	$15^{II} + Xy_r$	*
C. fulvus Goeze	32		*
C. globicollis Suffr.		$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. hypochoeridis L.		15^{II}	Barabas and Bezo[5]
C. hypochoeridis L.		$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. moraei L.	30	$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. ocellatus Drap.	30	$14^{II} + Xy_p$	*
C. ochroleucus Steph.	30	$14^{II} + Xy_p$	*
C. octopilosus Baly	32	$15^{II} + Xy_p$	Sharma and Sood[6]
C. oppositus Jac.	30	$14^{II} + Xy_p$	Sharma and Sood[6]
C. primarius Har.	40	$19^{II} + Xy_p$	*
C. quadruplex Newn.		$11^{II} + Xy_p$	Smith[3]
C. rugicollis Ol.		$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. sexmaculatus Ol.	28	$13^{II} + Xy_p$	*
C. sexpunctatus L.	16	$7^{II} + Xy_p$	Takenouchi and Shiitsu[7]
C. sexpustulatus Vill.	30	$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. sexsignatus F.	30	$14^{II} + XY$	Kacker[8]
C. sulphureus Ol.		$15^{II} + Xy_p$	*
C. triangularis Hope	32	$15^{II} + Xy_p$	Sharma and Sood[6]
C. venustus F.		$c12^{II}$	Smith[9]
C. violaceus Laich.		$14^{II} + Xy_r$	Alegre and Petitpierre[2]
C. vittula Suffr.	28	$13^{II} + Xy_p$	*
C. indet. sp.	30	$14^{II} + Xy_p$	Yadav[4]
C. indet. sp.	30	$14^{II} + Xy_p$	Yadav[4]

*Present paper.



Figures 1–5. Chromosomes of *Pachybrachis*. Metaphases I of *P. petitpierrei* (1), *P. catalonicus* (2) and *P. azureus* (3) with $7^{II}+Xy_r$. The Xy_r is arrowed. Spermatogonial metaphases of *P. catalonicus* (4) and *P. azureus* (5) with 2n=16 chromosomes. Figures 3 and 5, × 2000; others × 2500.



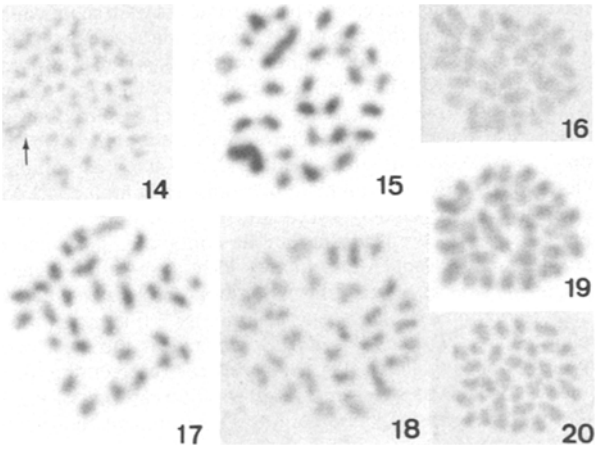
Figures 6–13. Chromosomes of *Cryptocephalus*. Metaphases I of *C. primarius* with $19^{II}+Xy_r$ (6), *C. vittula* $13^{II}+Xy_p$ (7), *C. sulphureus* $15^{II}+Xy_p$ (8), *C. crassus* $15^{II}+Xy_r$ (9), *C. bipunctatus* $14^{II}+Xy_r$ (10), *C. ocellatus* $14^{II}+Xy_p$ (11), *C. ochroleucus* $14^{II}+Xy_p$ (12) and *C. sexmaculatus* $13^{II}+Xy_p$ (13). The arrow shows the sex bivalent and the arrowhead points to the large autosomal bivalent of *C. vittula* and *C. sexmaculatus*. All figures ×2500, except figures 6 and 13, ×2000.

somes, the modal number for the *Cryptocephalus* as we pointed out before[2]. Nevertheless, most of the species not having the modal value have karyotypes of numbers very close to it, with 2n=28 or 2n=32 chromosomes (table 2). The origin of the two karyotypes with 2n=28 chromosomes, those of *C. sexmaculatus* and *C. vittula,* can be accounted for by a centric fusion between two autosomes, giving rise to the large chromosome pair found in both of them. Only three species of *Cryptocephalus* differ broadly in number in respect of the modal value and among them *C. primarius,* studied here, with 2n=40 chromosomes, has the highest number so far encountered in this genus.

A comparison between the karyotypes of *Cryptocephalus* and *Pachybrachis* species easily reveals great differences of chromosomal sizes, almost always small in the former and mostly of medium size in the latter. However, the rough measures of total complement length, taken from spermatogonial metaphases, have given similar values close to 35–40 µm in the species of both genera. Since current taxonomy places *Pachybrachis* as a more primitive genus than *Cryptocephalus*[10,11], the karyological derivation of the latter chrysomelids could be tentatively explained through a series of chromosomal fissions, from the 16-chromosome karyotype of *Pachybrachis* to the modal 30-chromosome set of *Cryptocephalus* species. Then, the gross chromosomal evolution of *Cryptocephalus* could possibly have occurred from the basic value by increases and decreases, giving rise to such extreme karyotypes as those with 2n=40 and 2n=16 chromosomes, respectively. Besides that, the additions or deletions of chromatin, probably heterochromatin, can also be involved in the karyological differentiation of some of the 30-chromosome species as we reported before[2].

The constancy of number and main chromosomal features of *Pachybrachis* and the relatively slight numeric variation found among the species of *Cryptocephalus* are presumably conditioned by the rather high capacity for active dispersal common to almost all of these flying chrysomelids. This characteristic would undoubtedly reduce the chances for fixation of any chromosome mutation, and would affect consequently the rates of chromosomal divergence of these species, as we have discussed for beetles elsewhere[12]. Both *Pachybrachis* and *Cryptocephalus* are polyphagous genera on a wide array of host plants[13], but many species show rather strict oligophagous tendencies at least in Palearctics. These feeding choices might provide a clue for understanding the high number of species which have appeared in the evolution of both genera with very small or even undetected chromosomal changes.

The prevalent male sex-determining systems of Cryptocephalinae are the distally associated sex-chromosomes, Xy_r, and the 'parachute' type, Xy_p. The first is found in all but one species of *Pachybrachis* and in about 45% of those of *Cryptocephalus,* whereas the second occurs in all the remaining except for a unique XY species, which has both male sex-chromosomes of large size instead of a small *y*-chromosome, the common rule in beetles. The Xy_p is generally considered the most frequent and primitive sex-determining system in the coleopterans[14], and the same probably holds true for the Cryptocephalinae, but in the present state of knowledge it is difficult to ascertain whether the



Figures 14–20. Chromosomes of *Cryptocephalus*. Spermatogonial metaphases of *C. primarius* with 2n=40, the X-chromosome arrowed (14), *C. vittula* 2n=28 (15), *C. crassus* 2n=32 (16), *C. ocellatus* (17) and *C. ochroleucus* (18) with 2n=30, *C. sexmaculatus* 2n=28 (19), and *C. fulvus* 2n=32 (20). Note the large autosome pair of *C. vittula* and *C. sexmaculatus*. Figures 14, 16 and 20 ×2000, others ×2500.

Xy_p or the Xy_r is the most primitive sex-determining system of the subfamily. Anyhow the Cryptocephalinae differ strikingly in this respect with regard to their most closely related subfamilies, such as the Megalopodinae and Clytrinae, in which unpaired sex chromosomes, X+y formula, seem to be the rule in the few investigated species of the former[15] and in several species of the latter[14,16]. In this sense, the Cryptocephalinae have kept more archaic sex-determining systems than those of Megalopodinae and Clytrinae, in spite of being more advanced on morphological grounds. Therefore, other chromosomal characteristics like the low number, 2n=20 in Megalopodinae[15] and 2n=20–24 in most Clytrinae[14,16], are probably better karyological hints of primitivism than the sex-determining systems.

1 To whom correspondence should be addressed.
2 Alegre, C., and Petitpierre, E., Experientia *38* (1982) 774.
3 Smith, S. G., Can. J. Genet. Cytol. *2* (1960) 66.
4 Yadav, J. S., Res. Bull. Panjab Univ. Sci. *22* (1971) 259.
5 Barabás, L., and Bežo, M., Biológia, Bratisl. *34* (1979) 845.
6 Sharma, G. P., and Sood, V. B., Nat. Acad. Sci. Lett. *1* (1978) 351.
7 Takenouchi, Y., and Shiitsu, T., Kontyû *40* (1972) 297.
8 Kacker, R. K., Ph. D. thesis, Banaras Hindu University, Varanasi, India, 1971.
9 Smith, S. G., Heredity *7* (1953) 31.
10 Clavareau, H., in: Coleopterorum catalogus, vol. 24, pars 53. Eds W. Junk and S. Schenkling. Berlin 1913.
11 Seeno, T. N., and Wilcox, J. A., Entomography *1* (1982) 1.
12 Petitpierre, E., 17th int. Congr. Ent., Abstract vol., (1984) 391.
13 Jolivet, P., Acta zool. path. antverp. *70* (1978) 167.
14 Smith, S. G., and Virkki, N., in: Animal Cytogenetics, vol. 3, Coleoptera, Insecta 5. Ed. B. John. G. Borntraeger, Berlin 1978.
15 Virkki, N., Hereditas *98* (1983) 209.
16 Petitpierre, E., Unpublished results.

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Identification of a new predaceous stink bug pheromone and its attractiveness to the eastern yellowjacket[1]

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Summary. Males of *Podisus fretus* (Hemiptera: Pentatomidae) release a long-range attractant pheromone containing linalool (49.0%), (*E*)-2-hexenal (34.5%), benzyl alcohol (12.0%), nerolidol (2.0%), α-terpineol (1.1%), and traces of several other compounds. The eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae), is attracted to artificial pheromones for *P. fretus* and for the sympatric species, *Podisus maculiventris*.
Key words. Pheromone; kairomone; semiochemical; attractant; *Vespula maculifrons; Podisus fretus;* Hemiptera; honeybee.

Foraging workers and queens of the eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae), are attracted to the pheromone of the predaceous spined soldier bug, *Podisus maculiventris* (Hemiptera: Pentatomidae)[2]. Individual components of the bug's pheromone (table 1) were unattractive to this wasp in 1983 field tests, but 1:1 mixtures of either (*E*)-2-hexenal and α-terpineol or (*E*)-2-hexenal and linalool were as attractive to yellowjacket workers as the entire pheromone in 1982 and 1983 tests[2]. Linalool constitutes less than 1% of the spined soldier bug pheromone (table 1)[3], suggesting that *V. maculifrons* is not specifically attracted to the pheromone of *P. maculiventris*[2]. However, we now report the discovery of a second *Podisus* species in our study area, provisionally identified as *P. fretus*, whose major pheromone components are (*E*)-2-hexenal and linalool. In addition, we have collected yellowjackets, honeybees, and *P. fretus* adults in pheromone-baited traps deployed in coniferous and deciduous forests for an entire season to more closely examine the chemical relationship between *Podisus* pheromones and yellowjackets.

Methods and materials. On 8 April 1984 a large dark brown female pentatomid was captured on the outside of a trap baited with *P. maculiventris* artificial pheromone. The female laid eggs, the resulting larvae were reared on *Tenebrio molitor* (Coleoptera: Tenebrionidae) pupae[4], and the first adults emerged 37 days after oviposition. The large dorsal abdominal pheromone glands were dissected from 1-week-old males[4], and the methylene chloride extracts were analyzed by gas chromatography/mass spectrometry (GC/MS) (Finnigan 4510) using a 30-m fused silica bonded methyl silicone capillary column (0.25-mm ID; 0.1-μm phase film; DB-1™; J & W Scientific, Rancho Cordova, CA), temperature programmed from 45 °C (isothermal for 2 min) to 240 °C at 15°/min. Compounds were identified by comparison of their electron impact mass spectrum (MS) to the published MS and/or the MS of authentic standards (nerolidol and (*E*)-2-hexenal from Bedoukian Research Inc, Danbury, CN; *cis*- and *trans*-piperitol from PCR Research Chemical Inc., Gainesville, FL; the remaining compounds from Aldrich Chemicals, Milwaukee, WI). All compound identifications were confirmed by comparison of the GC retention of the natural product to that of the standard using a Varian 3700 GC with a 15-m DB-1 capillary column, under isothermal conditions. Percentages of compounds in gland extracts were determined by GC analysis using a Shimadzu C-R3A peak area integrator.

Artificial pheromone blends for *P. fretus* and *P. maculiventris* were field tested during 1984 and 1985 in coniferous and deciduous forest tracts surrounding a 9.7-ha pasture at the Beltsville