different site from that of C3a, a similarly derived cleavage product of C3 (*12*). Fragment C5b can also participate in the chemotaxis of PMN's when it is complexed with C6 and C7 (*13*). A role for a C5-derived product, possibly C5b, has also been demonstrated in the opsonization of baker's yeast particles (*9*). Various functional activities of C5 products probably reflect different individual sites on the C5 molecule (*9, 14*). Characterization of the biologically active material in the milks may, therefore, contribute significantly to the characterization of the opsonically active fragment of human C5.

2) The identification of a biologic activity in milks with the potential for enhancing the inflammatory response has significance in a variety of human nutritional deficiency states. Preliminary characterization of the opsonically active bovine milk fraction by Sephadex G-200 chromatography showed no activity in the casein micelle complex (molecular weight 10^8). Heat-stable opsonic activity was found between the lactalbumin and lactoglobulin peaks. Lactalbumin lost opsonic activity and was denatured in highly acid *p*H ranges, suggesting that the opsonically active fraction resides in the lactoglobulin fraction, with a molecular weight of approximately 36,000. Effects of the various antiserums upon this partially purified isolated product are identical to those shown with whole bovine milk.

3) The identification of the material in nonanimal milks (that is, soy) suggests a wide biologic role for this molecule. Precedents exist for such relationships, for example, with the glycoprotein blood group antigens which are found throughout the animal and plant kingdoms. Complete characterization of the opsonically active milk fraction should permit exploration of this point.

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6. Previous data show that maximum yeast opsonic activity is present in serum concentrations of 10 percent or greater. When serum is used in concentrations greater than 50 percent in the yeast assay, one often finds inhibitory activities. We, therefore, use 10 percent serum as the standard positive control. Milk preparations at 90 to 100 percent had opsonic activities equivalent to 10 percent serum. Further studies will be necessary to determine the nature of these differences.
7. Three entirely different antiserums were used in these studies. (i) Antiserum to highly purified human C5 was raised in C5-deficient mice (strain A/HeJ) as described by U. R. Nilsson and H. J. Müller-Eberhard [*J. Exp. Med.* **125**, 1 (1967)]. (ii) Antiserum to human C5 raised in goats was obtained from Meloy Laboratories. (iii) Goat antiserum to human C5 was also obtained from Behring Diagnostics. Prior to use in the opsonic assays, each antiserum was shown to have a single immunodiffusion band directed against either highly purified human C5 or whole human serum.
8. These antiserums were obtained commercially from Meloy Laboratories, Behring Diagnostics, or Cordis Laboratories. In most cases, two sources of antiserums were used for each experiment. Each of the antiserums was added to milk in a range of final concentrations from 25 to 80 percent.
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Cleptoparasitism and Odor Mimetism in Bees: Do *Nomada* Males Imitate the Odor of *Andrena* Females?

Abstract. *Identical chemical compounds are present in the Dufour gland secretion of female* Andrena *bees and in the cephalic secretion of many male* Nomada *bees. Females of* Nomada *parasitize the nests of* Andrena. *Many* Nomada *species confine their attacks to a single host species. In two such host-parasite pairs,* Andrena haemorrhoa–Nomada bifida *and* Andrena carantonica–Nomada marshamella, *all*-trans *farnesyl hexanoate was found to be the totally dominant component in respective secretions. In two other pairs,* Andrena helvola–Nomada panzeri *and* Andrena clarkella–Nomada leucophthalma, *geranyl octanoate is the major component. This pairwise odor correspondence is discussed in relation to critical points of contact in the life cycles of host and parasite, male and female.*

In an investigation of biologically active volatile chemical compounds in bees of the genus *Andrena* F. we have found all-*trans* farnesyl hexanoate or geranyl octanoate to be the dominant component in the female Dufour gland secretion. These compounds are included in the nest odor. In the cephalic secretion of many male *Nomada* bees, which are cleptoparasites (nest parasites) mainly of *Andrena*, the same chemicals were found to be dominant. We believe that this finding will be of some importance for understanding the evolution of strategies that cleptoparasites use to gain entry into host nest.

The bee genus *Andrena* is found in the Holarctic and African regions and is often represented by populations rich in individuals. Most species of *Andrena* are solitary, some are communal (*1*). The females build their nests, consisting of a main burrow with short lateral tunnels, in the ground. At the end of each lateral tunnel is a nest cell coated on the inside with a hydrophobic lining. This coating is secreted from the Dufour gland located in the abdomen of the female (*2*). After the female has stocked the cell with a food supply of pollen and nectar and has laid an egg on top of it, the cell is sealed.

Species of *Nomada* parasitize mainly species of *Andrena* but also species in other genera of bees (*3*). Each species confines its attacks predominantly to a single host species or a group of closely related species. The *Nomada* female first locates *Andrena* nests from visual and chemical cues (*4*), and later, she lays an egg in a nest cell prepared by the host. The *Nomada* larva kills the host egg and consumes its food supply (*4*). In spite of the harm caused by the *Nomada* larva in the nest of the *Andrena*, an encounter between females of the two species in or just outside the nest causes no aggressiveness (*5*). The two females show no resemblance in general appearance in either color or pubescence (Fig. 1).

Members of the two populations of the host-parasite pair also meet in another situation. *Andrena* males make route flights (patrolling flights) (*6*) in habitats that are specific for different *Andrena* species. The males aggregate in a certain part of this habitat, where the flight paths are marked by odor points perfumed with volatile secretion from the cephalic glands (*7*). In species nesting in aggregations, the nest areas are patrolled by the males (*8*). The *Nomada* males make route flights in the same localities as the males of their host species. We have often observed *Nomada* males to join groups (often the more numerous ones) of *Andrena* males following certain flight paths.

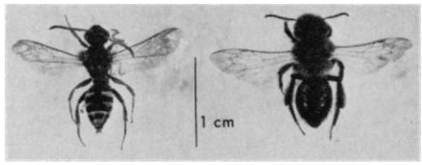

Fig. 1. Females of *Nomada marshamella* (left) and *Andrena carantonica* (right).

We have analyzed (*9*) the composition of abdominal (Dufour gland) and cephalic (mandibular gland) volatile secretions of *Andrena* and *Melitta* (*10*) bees. Esters of relatively low volatility have been found to be the major component in the Dufour gland secretion of 19 *Andrena* species (*11*). Of these, 16 contain all-*trans* farnesyl hexanoate and 3 have geranyl octanoate in large amounts (in quantities on the order of milligrams per individual). In two species of *Melitta*, several esters were found in the Dufour gland secretion with octadecyl butyrate as the dominant component (*12*). The cephalic secretion of 14 species of *Andrena* have also been analyzed (*13*). They are complex, species-specific mixtures; the secretions of males and females are of the same composition in most cases.

The parasitic bees generally have small Dufour glands. These glands have not been analyzed in *Nomada*. On the other hand, cephalic secretions of males and females have been studied in eight species (*14*) (Table 1). Both sexes produce relatively large amounts of cephalic secretions (on the order of 1 mg per individual). Contrary to the finding in *Andrena*, there are few similarities between males and females of the same species. In five species, the male cephalic secretion was dominated by a component identical to that from the Dufour gland in the female of their main host species (*Andrena* sp. or *Melitta* sp.) (*15*) (Table 1). Cephalic secretions of *Nomada* females have been studied only to ascertain any similarity to the cephalic secretions of the *Nomada* males or the Dufour gland secretions of *Andrena* or *Melitta* females. Not one of the analyzed cephalic secretions from *Nomada* females contained any farnesyl hexanoate (F6), geranyl octanoate (G8), or octadecyl butyrate (OD4). A typical capillary gas chromatogram showing the volatile composition of the cephalic secretion of a single *Nomada marshamella* male and of the Dufour gland secretion of *Andrena carantonica* are given in Fig. 2, A and B, respectively.

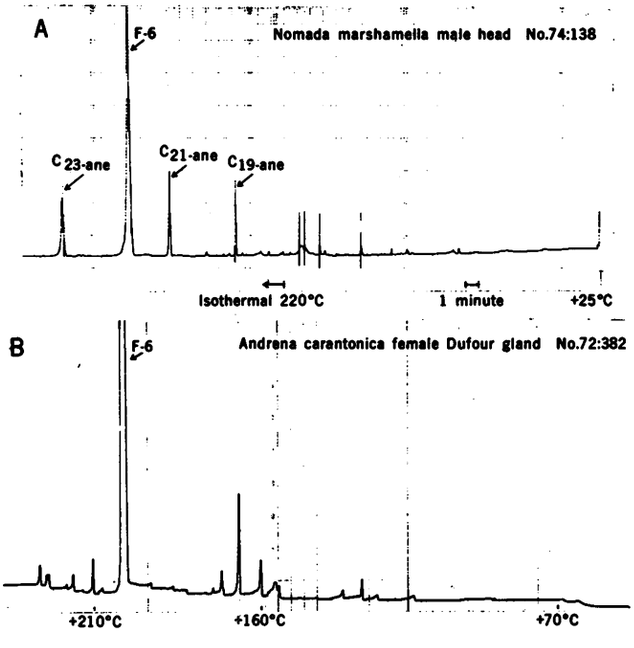

Fig. 2. (A) Capillary gas chromatograms of (A) the volatile cephalic secretion from N. *marshamella* (male) and (B) the volatile secretion from the Dufour gland of *A. carantonica* (female).

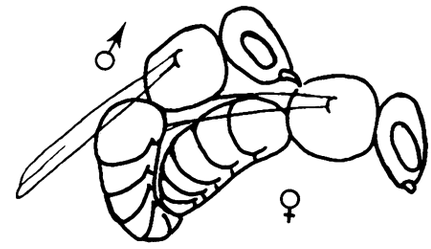

Fig. 3. Schematic drawing of the relative position of *Nomada* female and male in copula.

The chemical correspondence found in five host-nest parasite pairs may have significance for the coexistence of host and nest parasite. For those pairs of species that exhibit this chemical agreement, the relationships between *Andrena* female and *Nomada* male and female can be interpreted as follows.

Andrena females and males (the males at least in species nesting in aggregations) are attracted by nest site odor (*8*). Farnesyl hexanoate or geranyl octanoate (F6/G8) is a prominent part of this odor. *Nomada* females are also directed in part by chemical cues when they search for host nests. It is therefore likely that they are guided by F6/G8 emitted by the *Andrena* female. *Nomada* females encounter this odor also during mating, because it is emitted in the male cephalic secretion. It may be assumed that the same sensilla and neural circuitry functions in these two different behavioral phases of the female life cycle, expressing "neural parsimony" (*16*).

The host *Andrena* and the parasite *Nomada* are both bees (superfamily Apoidea), but they belong to different families, the taxonomic relationship of which is not considered to be close (*1*). This relationship suggests a long-established coexistence of *Andrena* and *Nomada* (*17*), with plenty of time available for *Nomada* to evolve a signal system adapted to that of *Andrena*. During copulation, the volatile secretion of the *No-*

Table 1. Main components in the volatile secretion of parasite (*Nomada*) and host (*Andrena* or *Melitta*). Main components not identified are indicated by class of compound. Abbreviations: F6, farnesyl hexanoate; OD2, octadecyl acetate; OD4, octadecyl butyrate; and G8, geranyl octanoate.

Parasite species	Cephalic glands (*Nomada*)		Dufour gland (*Andrena*) Female	Host species or subgenus
	Female	Male		
N. bifida	Sesquiterpenes	F6	F6	*A. haemorrhoa*
N. flavoguttata	Hydrocarbons	OD2	F6	*Micrandrena*
N. flavopicta	Hydrocarbons	OD4	OD4	*M. leporina*
N. goodeniana	Straight chain aliphatic	Sesquiterpenes	F6	*Melandrena*
N. leucophthalma	Hydrocarbons	G8	G8	*A. clarkella*
N. lineola	Farnesal isomers	Farnesal isomers	F6	*Plastandrena*
N. marshamella	Hydrocarbons	F6	F6	*A. carantonica*
N. panzeri (*20*)	Straight chain aliphatic	G8	G8	*A. helvola*

mada male cephalic glands is emitted and presumably sprayed onto the female partner (Fig. 3). Gas chromatographic and mass spectrometric data indicate that this is the case. The low volatility of F6/G8, and perhaps also the waxy coating and the structure of the cuticle, make these compounds adhere to and be emitted from the *Nomada* female for a long period of time. The F6/G8 may be the signal that establishes the nonaggressive relationship (*18*) between females of *Andrena* and *Nomada*. The perfuming of the female by the *Nomada* male might make the signaling more effective than if the compounds were produced by the *Nomada* female herself. If females perfumed with F6/G8 have advantages over unperfumed females or females perfumed with other compounds, the males producing F6/G8, and also this signal system, will be evolutionally favored. Males perfuming females during copulation have been reported in butterflies (*19*), who transfer a substance that acts as an antiaphrodisiac signal.

The chemical-physiological-ethological link between *Andrena* or *Melitta* (hosts) and *Nomada* (parasite) is supposed to be mediated partially through precise chemical compounds. These are volatile substances influencing behavior through the olfactory sense. In this case, they constitute a liaison among three elements in the biocenocis and facilitate their coevolution. The possible advantage of transfer of volatile compounds from male to female *Nomada* (over the female's producing these substances herself) presumably arises by binding the parasite male closer to the relationship between the host and the parasite females.

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5. We have neither observed any aggression between *Nomada* and *Andrena* females nor seen any such reports in the literature. About other genera of solitary bees, contradictory statements about conflicts between host and cleptoparasite females have been made. The parasite females have often been observed to sit outside the host nest waiting for the host female to leave, which has been interpreted as an indication that the host female would defend her nest. However, the parasite must wait for an appropriate moment in the nest cell construction for her egg laying, which must cause her to check the stage of development of the nest after each occasion the host female has been in the nest. From social bees there are also reports of both aggressive and nonaggressive relationship between host and cleptoparasite. H. Friese [*Zool. Jahrb. Abt. Syst. Oekol. Geogr. Tiere* **3**, 847 (1888)] postulated concerning the nonaggressive relationship of *Andrena* and *Nomada* females, about the odor of *Nomada*. "Vielleicht liegt in diesem Individual-geruch das freundschaftliche Verhältnis begründet." A → W. M. Wheeler [*Proc. Am. Philos. Soc.* **58**, 1 (1919)] discussed the significance of odor in the nest parasite relation to the host.
6. A. Haas, *Z. Tierpsychol.* **17**, 402 (1960).
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10. The behavior of *Melitta* species is essentially the same as that of the *Andrena* species.
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13. ———, *Comp. Biochem. Physiol. B.* **55**, 179 (1976); in preparation.
14. G. Bergström and J. Tengö, in preparation.
15. The chemical correspondence between *Andrena* and *Nomada* is constituted through esters of isoprenoid alcohols, geraniol, and farnesol. The compound in the *Melitta-Nomada* relationship is an ester of a fatty alcohol, octadecanol. The isoprenoid esters and octadecyl butyrate each represent separate biosynthetic pathways.
16. K. D. Roeder, *Nerve Cells and Insect Behavior* (Harvard Univ. Press, Cambridge, Mass., 1967).
17. R. R. Askew, *Parasitic Insects* (Elsevier, London, 1971).
18. Most bees defend their nests. The significance of odor in aggressive relationship between host and parasite have been demonstrated by S. W. T. Batra [*J. Kans. Entomol. Soc.* **38**, 367 (1965)] concerning a mutillid wasp and a halictid host bee, *Lasioglossum zephyrum*, and by E. Ordway [*J. Kans. Entomol. Soc.* **37**, 139 (1964)] concerning the bee *Sphecodes pimpinellae* and its two host species of the halictid bee *Augochlorella*. The visit of a female *S. pimpinellae* to a host nest causes a change in host bee behavior and has an inhibitory effect on the nesting activity, lasting for a few weeks. Not leaving an unfamiliar smell in the host nest must be advantageous to *Nomada* females with the F6/G8 odor.
→ L. E. Gilbert, *Science* **193**, 419 (1976).
20. *Nomada panzeri* is closely related or synonymous to *N. ruficornis*. The *N. ruficornis* group is not clear taxonomically. Most authors regard *A. helvola* as one of the hosts of *N. ruficornis*.
21. Supported by the Swedish Natural Science Research Council, the Ekhaga Foundation, the Trygger Foundation, and the Axel and Margaret Ax: son Johnson Foundation. We thank B. Kullenberg and T. Norin for stimulating discussions and constructive criticism of the manuscript. We also thank M. Schwarz for determination of most of the *Nomada* bees.

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Hypoxanthine Phosphoribosyltransferase: Two-Dimensional Gels from Normal and Lesch-Nyhan Hemolyzates

Abstract. *Immunoprecipitated hypoxanthine phosphoribosyltransferase (HPRT) from hemolyzates displays two major spots after two-dimensional polyacrylamide gel electrophoresis. HeLa cells or human lymphoblasts display only a single HPRT spot located at the same position as the most basic of the hemolyzate HPRT spots. This suggests that the most basic spot is the form initially synthesized, and the more acidic hemolyzate HPRT spot (a pseudoisozyme) is probably derived from the first by an age-related modification (for example, deamidation). The HPRT pattern of the hemolyzate from a Lesch-Nyhan patient was shifted to a more basic isoelectric* p*H, implying the mutation of a structural gene.*

The purine salvage enzyme hypoxanthine phosphoribosyltransferase (HPRT, E.C. 2.4.2.8) catalyzes the synthesis of inosine or guanosine monophosphate from 5-phosphoribosyl-1-pyrophosphate and hypoxanthine or guanine. Clinically, HPRT-deficiency is associated with the X-linked Lesch-Nyhan syndrome (*1*). This disorder is characterized by mental retardation, self-mutilation, and high concentrations of uric acid in the blood and urine. Patients with Lesch-Nyhan syndrome usually display virtual absence of HPRT activity in their erythrocytes. Immunological studies indicate that these erythrocytes sometimes contain nonfunctional HPRT protein which cross-reacts with antiserums prepared against erythrocyte HPRT from normal individuals (*2*, *3*).

We have described the analysis of radioisotope-labeled normal and mutant forms of HPRT from tissue culture cells by immunoprecipitation and two-dimensional polyacrylamide gel electrophoresis (*4*). In this report, we describe the results of a similar analysis of unlabeled HPRT in red blood cell hemolyzates.

The immunoprecipitation of HPRT protein from hemolyzates was conducted according to a modification of the double antibody procedure previously described (*2*). All steps were performed at 0° to 4°C. Hemolyzates were prepared as described by Arnold and Kelley from outdated normal blood obtained from a blood bank (*5*). The Lesch-Nyhan hemolyzate was from patient E.S. (*6*). A 30-μl sample of hemolyzate was mixed with 90 μl of enzyme buffer (20 m*M* KCl, 6 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.5 m*M* dithiothreitol, and 20 m*M* tris-hydrochloride, *p*H 7.8)