Sex Pheromones: Abolition of Specificity in Hybrid Bark Beetles

Abstract. *Specificity of sex pheromones maintains breeding isolation among three closely related species of spruce-infesting* Ips. *Hybrids produced in the laboratory were intermediate to the parent species in both attractiveness and response. Pheromones and pheromone receptor types in the hybrids are probably mixtures of those of the parent species.*

The spruce-infesting bark beetles (Scolytidae) *Ips amiskwiensis* G. Hopping and *I. borealis* (Eichhoff) are sympatric along the eastern edge of the Canadian Rockies (*1*). Near Banff, Alberta, these species commonly infest the same host tree, but I have been unable to find them inhabiting the same gallery systems. *Ips pilifrons* Swaine infests spruce in the southern Rocky Mountains and meets *I. amiskwiensis* in the vicinity of the Grand Tetons, Wyoming (*2*).

Although *I. amiskwiensis* will produce fertile hybrids with *I. borealis* and *I. pilifrons* in forced laboratory pairings, putative hybrids are rare in nature. Introgression is presumably averted by specificity of sex pheromones (*3*). I have found support for this hypothesis in field and laboratory tests of females' responses to pheromones produced by various pure and hybrid males. The same tests indicate that hybrids produce a mixture of pheromones and inherit pheromone receptor types of both parent species.

For each laboratory test, 36 males (12 each of three kinds) were induced to bore into a freshly cut spruce bolt (*4*) in a 6 by 6 Latin square design. Each kind of male was represented twice in every row and column. After males were allowed 2 days to excavate nuptial chambers, 72 females of one kind (occasionally less than 72 were available) were released on the infested bolt in a cage made by joining two 1-gallon Sealright (*5*) food cartons. Four days later the bark was carefully stripped from the log and the number of females in, or constructing egg galleries from, each nuptial chamber was recorded. Nuptial chambers rather than males were considered in the assessment of the relative attractiveness of the kinds tested; occasionally males failed to become established, whereas others abandoned the initial nuptial chamber to construct a second. Correct identification of such males was assured by prior marking of each kind with a different lacquer.

In the first type of field test, small logs, each previously infested with five males of one kind, were placed 5 m apart in a Latin square. Each kind appeared once in every row and column. After 10 days, results were assessed as in laboratory tests. In the second type of field test, logs, each containing 40 males of one kind, were placed in individual greenhouse cages and responding beetles were collected in a tray of water under a window trap (*6*).

In 56 of 57 laboratory tests, more pure females responded to males of their own species than to males of other species. Based on attraction to males of its own kind as index 100, attraction indices (*7*) for *I. amiskwiensis* females to *I. pilifrons* and *I. borealis* were 37 and 19, respectively (Table 1). Indices for reciprocal tests were 11 and 15. In field tests, *I. amiskwiensis* was very slightly, if at all, attracted to other species (Table 2).

Pure females generally responded to hybrid males at a level intermediate to the response to males of the parent species. Attraction indices for *I. pilifrons* and *I. borealis*—to the pure species and their F_1 and B_1 progeny with *I. amiskwiensis*—are clearly in the same order as their blood relationships. For example, the responses of female *I. pilifrons* to male *pilifrons*, B_1 *p* (*a-p*) (*8*), F_1 *a-p*, B_1 *a* (*a-p*), and *I. amiskwiensis* were 100, 35, 27, 13, and 11, respectively (Table 1). *Ips amiskwiensis* females also responded in order of blood relationship with *I. borealis* and backcrosses in both laboratory (Table 1) and field tests (Table 2, test 1). However, the ability of *I. amiskwiensis* to discriminate apparently broke down in laboratory tests invo'ving its F_1 hybrid with *I. pilifrons*. Furthermore, it did not differentiate between the B_1 *p* (*a-p*) and *a* (*a-p*) in the laboratory or in the field (Table 2, tests 2 and 3).

Hybrid *a-p* females were slightly more attracted to males of their own kind than to those of the two parent species (Table 1). Backcross *p* (*a-p*) and *a* (*a-p*) females were attracted strongly to males of the backcross species, less to males of their own kind, and least to males of the other parental species. F_1 *a-b* were insufficient in number for inclusion in tests. Backcross female *b* (*a-b*) responded in the manner just described. However, the *a* (*a-b*) females were attracted nearly equally

Table 1. Attraction indices (*8*) and number of tests (italics) for *Ips amiskwiensis* (*a*), *I. pilifrons* (*p*), *I. borealis* (*b*), and F_1 and backcross (B_1) hybrids, determined in the laboratory. NT, not tested.

Females	Male pheromone				
	a	*a* (*a-p*)	*a-p*	*p* (*a-p*)	***p***
I. amiskwiensis	100-*8*	52-*2*	63-*4*	59-*2*	37-*8*
B_1 *a* (*a-p*)	189-*4*	100-*4*			82-*4*
F_1 *a-p*	78-*4*		100-*4*		89-*4*
B_1 *p* (*a-p*)	30-*4*			100-*4*	143-*4*
I. pilifrons	11-*8*	13-*4*	27-*3*	35-*5*	100-*10*
	a	*a* (*a-b*)	*a-b**	*b* (*a-b*)	***b***
I. amiskwiensis	100-*6*	42-*4*	NT	34-*3*	19-*5*
B_1 *a* (*a-b*)	94-*8*	100-*8*	NT		47-*8*
B_1 *b* (*a-b*)	52-*5*		NT	100-*5*	115-*5*
I. borealis	15-*5*	19-*5*	NT	74-*4*	100-*7*

Table 2. Attraction indices (*8*) of female *Ips amiskwiensis* to various pure and hybrid males determined in the field. Tests 1 and 2, females taken from nuptial chambers of males; test 3, females captured in traps. NT, not tested.

Test	Total taken (No.)	Male pheromone							
		p	*p* (*a-p*)	*a-p*	*a* (*a-p*)	*a*	*a* (*a-b*)	*b* (*a-b*)	*b*
1	71	10	15	66	NT	100	NT	21	9
2	90	0	46	45	39	100	37	1	0
3	100	16	69	NT	66	100	62	0	NT

to males of the same kind and to *I. amiskwiensis*.

From these tests it is clear that specificity of sex pheromones tends to prevent natural hybridization between *I. amiskwiensis* and *I. borealis* or *I. pilifrons*. Comparisons of laboratory and field tests with *I. amiskwiensis* suggest that specificity is greatest under field conditions and that greater discrimination is exercised in entering nuptial chambers (log tests) than in flying to the attractant (trap tests). However, natural hybrids which might occur could readily breed among themselves, or assimilate with either parent species.

Pheromones and pheromone receptors of hybrids theoretically could be new, the same as those of the parent species (either singly or in combination), or a combination of new and parent types. Receptors of pheromones in some insects have been shown to be quite specific; even isomers of the same compound often fail to evoke equivalent response (*9*). It is therefore unlikely that the genetic condition of hybrids results in new receptors which are spontaneously keyed to new pheromones. Rather, the parent pheromones and receptors are probably present in proportions similar to the degree of heterosis. If the olfactory response is a function of the number of individual receptors stimulated (*10*), the F_1 *a-p* females should be expected to respond highest to F_1 *a-p* males because the mixed pheromones of those males would excite the maximum number of the mixed receptors of the females. The preference of backcross females for the pheromone of the pure (backcross) species would also be predicted (*11*). Lack of discrimination by *I. amiskwiensis* between pheromones produced by male *p* (*a-p*) and *a* (*a-p*) and the partial breakdown in response specificity of this species in the presence of the F_1 *a-p* pheromone may be associated phenomena—the cause of which could come to light when the chemistry of the component pheromones is known.

G. N. LANIER

Canadian Forestry Service, Department of Fisheries and Forestry, Calgary, Alberta

References and Notes

1. G. R. Hopping, *Can. Entomol.* **97**, 159 (1965); *ibid.*, p. 193.
2. Based on recent collections by the author.
3. The male *Ips* selects the host tree, initiates the attack, and constructs a nuptial chamber in the phloem-cambium region. In so doing it discharges a pheromone which attracts both sexes, stimulates mass attack on the host selected, and induces females to enter the nuptial chambers and mate [R. F. Anderson, *J. Econ. Entomol.* **41**, 596 (1948); D. L. Wood and J. P. Vité, *Contrib. Boyce Thompson Inst.* **21**, 79 (1961)]. Cross attractiveness of sex pheromones has been demonstrated for several closely related allopatric bark beetles [J. P. Vité, R. I. Gara, H. D. von Scheller, *ibid.* **22**, 461 (1964); D. L. Wood and G. N. Lanier, unpublished data]. However, sympatric species are generally not cross attractive [R. C. Wilkinson, *Fla. Entomol.* **47**, 57 (1964); J. P. Vité, *Naturwissenschaften* **52**, 267 (1965)].
4. *Picea engelmannii*, 40 cm long and 10 to 20 cm in diameter.
5. Crown Zellerbach Ltd.
6. J. A. Chapman, *Can. Entomol.* **98**, 50 (1966); ——— and J. M. Kinghorn, *ibid.* **87**, 46 (1955).
7. Attraction index of *b* to *a*, for example, is calculated as follows: *b* per *a* nuptial chamber divided by *a* per *a* nuptial chamber times 100.
8. *I. amiskwiensis, a; I. pilifrons, p; I. borealis, b*; the progeny resulting from backcross (B_1) of *I. amiskwiensis–I. pilifrons* hybrids to *I. pilifrons* are designated *p* (*a-p*).
9. M. Jacobson, *Insect Sex Attractants* (Interscience, New York, 1967).
10. D. Schneider, *J. Insect Physiol.* **8**, 15 (1962).
11. The simplest genetic situation possible is single loci for pheromone and pheromone receptor type. Thus, the genotype for receptors of the F_1 *amiskwiensis-pilifrons* would be *ap* and those of the backcross to *pilifrons* would be ½ *ap* and ½ *pp*. The response of backcross females with the *ap* genotype is expected to be similar to that of the F_1. Those with the *pp* genotype should respond in a manner similar to *I. pilifrons*. If the response levels of the two genotypes predicted by the attraction indices in Table 1 are summed, it is clear that the *I. pilifrons* pheromone will provide the greatest aggregate attraction to backcross females. If more than one locus is responsible for determining receptor type, this preference of the B_1 to the backcross species should be accentuated. These approximations could be further complicated if more than one compound differs in the respective pheromones. The sex pheromone of *Ips confusus* (LeConte) consists of three compounds which act synergistically [R. M. Silverstein, J. O. Rodin, D. L. Wood, *Science* **154**, 509 (1966); D. L. Wood, R. W. Stark, R. M. Silverstein, J. O. Rodin, *Nature* **215**, 206 (1967)]. *Ips latidens* (LeConte), a primitive species in my judgment, was attracted to a combination of two of these compounds, but response was inhibited by addition of the third compound [D. L. Wood *et al.*, cited above].
12. I thank Dr. J. A. Chapman for review of the manuscript and G. A. Shofer for technical assistance.

3 February 1970; revised 14 May 1970

Scheie and Hurler Syndromes: Apparent Identity of the Biochemical Defect

Abstract. *Fibroblasts cultured from the skin of Scheie and Hurler patients are deficient in the same specific factor required for normal mucopolysaccharide metabolism.*

The Scheie syndrome, a rare genetic disorder of mucopolysaccharide (MPS) metabolism, resembles in some ways the better-known Hurler syndrome (*1, 2, 3*). Both diseases are characterized by increased amounts of two MPS's, chondroitin sulfate B and heparitin sulfate, in the urine (*4*). The patient's corneas are cloudy, and there are pathological changes of the skin and cardiac valves. However, the two disorders vary greatly in severity. Hurler patients have stunted growth and mental capacity and severe skeletal deformities; they rarely survive beyond age 10. By contrast, individuals affected with the Scheie syndrome have normal stature and lifespan, skeletal problems in the extremities only, and normal or superior intelligence.

A technique developed in this laboratory has made it possible to examine the biochemical relationships between the Hurler syndrome and other mucopolysaccharidoses. Because of an impairment in the mechanism of degradation of MPS, fibroblasts cultured from the skin of Hurler patients accumulate excessive amounts of radioactive chondroitin sulfate B when supplied with $^{35}SO_4^{2-}$ (*5*). Their characteristic patterns of ^{35}MPS accumulation and turnover can be converted to normal, however, if they are mixed with fibroblasts of genotype other than Hurler, or supplied with secretions of such fibroblasts (*6, 7*). The corrective "factor" in these preparations is a heat-labile macromolecular substance, probably a protein. Among the fibroblasts tested, those found to correct the defect of Hurler fibroblasts include cells derived from normal individuals, from patients with two closely related mucopolysaccharidoses (the Hunter and Sanfilippo syndromes), and from patients with several other genetic diseases. On the other hand, fibroblasts derived from Hurler patients do not correct each others' abnormal patterns. Thus, absence of a factor required for normal MPS metabolism is characteristic of Hurler cells and is the most specific defect reported so far in that disorder (*8*). Cells of the Hunter genotype likewise lack a specific factor, different from that in which Hurler cells are deficient (*7, 9*); lack of yet two other genotype-specific factors occurs in the Sanfilippo syndrome (*10*).

Fibroblasts derived from Scheie individuals resemble those from Hurler patients in that they do not secrete the factor required to correct the defect in