SEX PHEROMONE RESPONSES OF *CHORISTONEURA* SPP. AND THEIR HYBRIDS (LEPIDOPTERA: TORTRICIDAE)

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Abstract

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Field studies in Ontario, Oregon, and California on interspecific attraction and inhibition among the coniferophagous species of *Choristoneura* (the spruce budworm and its allies) confirmed specific pheromonal differences among the species. Of the three species that are mutually attractive and whose males are attracted by blends of *trans*- and *cis*-11-tetradecenal, *C. fumiferana*, *C. biennis*, and *C. occidentalis*, catches of *C. fumiferana* males in traps baited with *C. fumiferana* females were significantly reduced by the presence of females from a second group in which it is believed the females produce *trans*-11-tetradecenyl acetate. Catches of *C. occidentalis* males by *C. occidentalis* females were not affected in this way. F_1 hybrids and backcrosses between an aldehyde-producing species and an acetate-producing species produced females that were attractive to males of one or other of the parent species, although some females were not attractive to either. Individual females never attracted males of both parent species, and of those that were attractive, more were attractive to males of the aldehyde species than to males of the acetate species. The results suggest sex-controlled inheritance and expression.

Résumé

Des études effectuées sur le terrain en Ontario, en Oregon et en Californie sur l'attirance interspécifique et l'inhibition chez les espèces de *Choristoneura* (la Tordeuse des bourgeons de l'Epinette et ses alliés), se nourrissant de résineux ont confirmé des différences phéromonales entre les espèces. Des trois espèces qui s'attirent mutuellement, et dont les mâles sont attirés par des mélanges de *trans*- et *cis*-11-tétradécénal, *C. fumiferana*, *C. biennis* et *C. occidentalis*, les prises de *C. fumiferana* mâles dans des pièges appâtés avec des *C. fumiferana* femelles furent significativement réduites par la présence de femelles d'un second groupe dans lequel les femelles produiraient de l'acétate *trans*-11-tétradécényl. Les prises de *C. occidentalis* mâles attirés par les femelles de leur espèce ne subirent pas cette influence. Des hybrides F_1 et l'introgression entre espèces productrices d'aldéhyde et espèces productrices d'acétate ont donné des femelles attirant des mâles de l'une ou l'autre des espèces mères, quoique certaines femelles n'attirèrent ni l'un ni l'autre des mâles. Les femelles individuelles n'attirèrent jamais de mâles des deux espèces mères, et parmi celles qui furent attirantes, un plus grand nombre ont attiré des mâles de l'espèce aldéhyde que des mâles de l'espèce acétate. Les résultats portent à croire à une hérédité et à une expression contrôlées selon le sexe.

Introduction

Sex attractants of Lepidoptera (and by attractants we refer to those components of the sex pheromone communication system that cause males to fly toward the source) are for the most part highly species specific. A study of the sex pheromone communication system of closely related species and of the effects of hybridization is therefore of considerable interest in understanding speciation and the maintenance of species integrity. It is also of practical significance, since it aids in a fundamental understanding of the role of sex pheromones in mating behavior and provides clues as to which chemicals may be most effective in disrupting mating behavior, providing the economic entomologist with a method of regulating pest populations.

Although there have been numerous studies on pheromone response among closely related species, so far there have been only two reports on the effects of hybridization on

sex pheromone response, that of Lanier (1970) on bark beetles of the genus *Ips* and that of Grant *et al.* (1975) on tussock moths of the genus *Hemerocampa*. The spruce budworm complex of the genus *Choristoneura* provides a very convenient group of species for such studies since (*a*) there is considerable information on their biology, (*b*) sex attractants are known for several of them, (*c*) they hybridize readily in the laboratory, and (*d*) they provide a range of interrelationships including allopatric species and sympatric, synchronic species. The species we have concentrated on are *C. fumiferana, C. pinus, C. occidentalis, C. biennis, C. viridis,* and *C. orae* with additional observations on a seventh species tentatively identified as *C. subretiniana* (*sensu* Obraztsov 1962), a species feeding on *Pinus contorta* in northern California. Until 1953 the first six species were all included as "spruce budworm" under the name *C. fumiferana* (Clem.). In 1953 *C. pinus* was recognized as a distinct species (Freeman 1953) and in 1967 the other five species were recognized (Freeman 1967). We do not intend to discuss the validity of their specific status here; suffice it to say that these names do represent populations which can be distinguished both morphologically and geographically.

In a preliminary report (Sanders 1971*a*) it was shown that three of the species, *C. fumiferana, C. biennis,* and *C. occidentalis,* apparently share the same attractant, since females of all three species attracted male *C. fumiferana*. The other three species showed some affinity to each other: *C. orae* females attracted considerable numbers of *C. pinus* males, while *C. viridis* females attracted a small but still significant number of *C. pinus* males. Subsequently the major component of the natural attractant of *C. fumiferana* was identified as *trans*-11-tetradecenal (TDAL) (Weatherston *et al.* 1971) and synthetic material of 97% purity was shown to be attractive to male *C. occidentalis* and *C. biennis* (Sanders *et al.* 1974), confirming the earlier work. Later the related acetate, *trans*-11-tetradecenyl acetate (TDACET), was shown to be an attractant for male *C. viridis* (Sanders *et al.* 1974). TDACET also elicits a greater electroantennogram response from antennae of male *C. pinus* than other 14-carbon chain acetates, alcohols, and aldehydes (Roelofs *et al.*, pers. comm.; Grant, pers. comm.), and preliminary chemical analysis indicates that TDACET is present in washes from *C. pinus* females (Weatherston, pers. comm.; Roelofs, pers. comm.). However, synthetic TDACET has been field-bioassayed over a wide range of concentrations and in conjunction with numerous other chemicals, but few male *C. pinus* have been captured. TDACET is one of two known inhibitors of male *C. fumiferana* (Sanders *et al.* 1972); the other, *trans*-11-tetradecenol (TDOL), has been found in the abdominal tips of female *C. fumiferana* (Weatherston and Maclean 1974), the probability being that it is a precursor of TDAL, but that it is not released by a calling female. Subsequent investigations have shown that small traces of *cis*-11-tetradecenal (CDAL) (often present as impurities in synthetic TDAL) are essential for effective attraction of male *C. fumiferana* and male *C. occidentalis,* the optimum ratio being between 2% and 5% CDAL for *C. fumiferana* (Sanders and Weatherston 1976) with a different, as yet undetermined, ratio for *C. occidentalis* (Daterman, unpub. data).

The incidence of hybridization in the "wild" among the different species has not been extensively studied. A diligent search by Smith (1954) failed to find any evidence of hybridization between *C. fumiferana* and *C. pinus*. However, when the insects are restricted in cages, hybridization readily occurs (Smith 1953; Campbell 1967). The coniferophagous species of *Choristoneura* therefore present an opportunity to study not only multiple pheromone systems and the pheromone interactions of related species but also, through hybridization, the genetic mechanisms regulating the inheritance of pheromone production. We report here field trapping data using virgin conspecific females and virgin hybrid females.

Insect Stock and Rearing Methods

The origin of the insect material used is outlined below.

Ontario Trials

***C. fumiferana* and *C. pinus*.** All insects came from laboratory stock maintained in Sault Ste. Marie, Ont., since 1966, and supplemented each year by field-collected material to maintain population vigor.

***C. occidentalis*.** The original material was obtained from a collection made by D. Doidge of the Canadian Forestry Service near Lillooet, B.C., in July 1970 and was subsequently maintained by laboratory rearings in Sault Ste. Marie, Ont. It was supplemented in 1972 by material sent by F. Schmidt, USDA Forest Service, Pacific Northwest Forest Experiment Station, Corvallis, Oreg., from stock maintained by R. L. Lyon, USDA Forest Service, Pacific Southwest Forest Experiment Station, Berkeley, Calif. This stock originated from material collected in the field in 1965 and was supplemented by field collections in 1970.

***C. viridis*.** The original material was field collected by F. Schmidt in the Warner Mountains, California, in July 1970 and was subsequently maintained by laboratory rearings in Sault Ste. Marie, Ont. It was supplemented in the fall of 1971 by material from stock maintained by F. Schmidt that originated from field collections in the Warner Mountains in 1968 and 1969.

***C. orae* and *C. biennis*.** Material was field collected in July 1970 — *C. orae* by E. V. Morris of the Canadian Forestry Service near Kitimat, B.C., and *C. biennis* by W. Vanderwal of the Canadian Forestry Service near Canal Flats, B.C. It was maintained by laboratory rearings in Sault Ste. Marie, Ont.

***C. subretiniana*.** This material was field collected in 1974 by G. E. Daterman from the Modoc National Forest in the Warner Mountains in northern California, approximately 20 air miles (32 km) northeast of Alturas, Calif. The insects were collected as larvae from a nearly pure stand of *Pinus contorta* Dougl. A culture is currently being maintained in the Corvallis laboratory.

Western Trials

***C. fumiferana* and *C. pinus*.** All insects were obtained from the same stock used in the Ontario trials.

***C. occidentalis*.** The original material was obtained from 1971 and 1972 field collections by G. E. Daterman in the Hat Point area of northeastern Oregon (approximately 10 air miles (16 km) southeast of Imnaha, Oreg., Wallowa Whitman National Forest). A supplementary field collection was made in 1973 by C. G. Thompson, USDA Forest Service, Forestry Sciences Laboratory, Corvallis, Oreg., from the Wenatchee National Forest approximately 25 air miles (40 km) northwest of Ellensburg, Wash. All field collections were subsequently maintained by laboratory rearings in the Corvallis laboratory.

***C. viridis*.** The original material was obtained in 1971 and 1972 by G. E. Daterman from field collections in the Warner Mountains (southern Oregon) approximately 10 air miles (16 km) east-southeast of Lakeview, Oreg. Additional collections were made in 1974 from the Warner Mountains (northern California) approximately 25 air miles (40 km) northeast of Alturas, Calif. All field-collected *C. viridis* were maintained by laboratory rearings in Corvallis, Oreg. In 1973 these stocks were supplemented with laboratory-maintained material provided by F. Schmidt of the Corvallis laboratory. This material had also originated in the Warner Mountains region (see above under *Ontario Trials*).

***C. subretiniana*.** This material was from the collection used in the Ontario trials.

***C. orae* and *C. biennis*.** These species were not included in the western trials.

Hybrids (Ontario and Western Trials)

All F_1 hybrids were obtained from the stock maintained in Sault Ste. Marie by setting up the appropriate matings in January 1971 and 1972. The resulting second-instar hybrids were then stored for 20–25 wk at 5°C to satisfy diapause requirements as is customary in all laboratory rearings with the parent species. Thus the different F_1 hybrids were available in the summer of 1971 and 1972 when they were field-bioassayed in Ontario. Reciprocal crosses of *C. viridis* and *C. occidentalis* were also obtained from F. Schmidt for the 1972 field season. For the 1974 field

season backcrosses were obtained, as well as F_1 hybrids. First, F_1 hybrids were obtained from matings in July 1973, then in January 1974 these were backcrossed to the parent stock, and at the same time more F_1 hybrids were obtained. To ensure success during the earlier matings, males and females were set up in groups for mass matings. However, in the January 1974 matings individual males and females were placed together to assess their readiness to hybridize and the success of the various mating combinations.

All insects were reared on synthetic diet (McMorran 1965, modified by Harvey 1974) using techniques similar to those described by Grisdale (1970), between 21°C and 25°C and approximately 70% R.H. under a 17-h photoperiod. The insects were sexed as pupae and the separate sexes were transferred to separate rooms and held under a 17-h photoperiod in screened cages. The cages were sprayed with water each day.

Throughout the remainder of the paper specific names will be abbreviated as follows: *C. fumiferana* = *Fu*; *C. pinus* = *Pi*; *C. occidentalis* = *Oc*; *C. viridis* = *Vi*; *C. biennis* = *Bi*; *C. orae* = *Or*; and *C. subretiniana* = *Su*. In the designation of hybrids the female parent is named first, i.e., *FuPi* = a *C. fumiferana* ♀ × *C. pinus* ♂.

Methods and Results

1. *Success of Hybrid Matings*

The jack pine budworm, *Pi*, is largely sympatric with *Fu*, and the adult flight periods of the two species show some overlap, giving partial synchrony. Although hybrid matings (e.g., a brown male mated with a grey female) would be easy to detect, none has been recorded (e.g., Smith 1953); in view of the species specificity of the sex attractants perhaps none should be expected (Sanders 1971*b*). The situation in the west is more complex. Owing to the wide variety of colormorphs in most species, hybrids would be extremely difficult to detect and it is possible that natural hybrids are formed. When budworm adults are confined in small cages hybrid matings do occur and the potential for hybridization can be assessed by choice of mate (Smith 1954; Campbell 1967). In the experiment reported in this section, designed to assess the success of hybrid matings, the insects were given no choice; pairs of adult insects were placed together in 8-oz (227-ml) glass jars with a short sprig of balsam fir (*Abies balsamea* [L.] Mill.) for all females except *Pi*, for which jack pine (*Pinus banksiana* Lamb.) was used. Jars were closed with perforated screw lids. Each day water was sprayed on the foliage. Since the eggs hatch after 8 days, all needles bearing eggs were removed from the jars after 7 days and placed in petri dishes for emergence and for larvae to establish hibernacula following the technique of Stehr (1954). Females were then returned to the jars for a further week to complete oviposition. For each mating the number of eggs laid, the number hatching, and the number of second-instar larvae in hibernacula were recorded. The females were subsequently dissected and if a spermatophore was present the mating was deemed successful. An attempt was made to set up at least 20 matings for each combination; however, in this experiment the development of *Oc* was not synchronized with that of the other species, and few individuals were available. The results are given in Table I.

Among the conspecific matings the *Fu* matings showed a high rate of success both in the transfer of spermatophores and in subsequent fertility. The *Pi* matings were low on both counts, while the *Vi* showed low fertility following good mating success. The reasons for these differences are not clear; however, the success of the F_1 matings and backcrosses was good, and their subsequent fertility was high. The only instances in which there was less than 50% mating success or 50% egg-hatch involved *Pi* males; this was not surprising in view of the poor results from conspecific *Pi* matings. The number of second-instar larvae established in hibernacula is a function of female size, fertility, and larval survival. Not all the species are the same size, and some of the variation in the

final column of Table I can be attributed to the smaller size of *Pi* and *Vi* females. There is no clear evidence for lower survival in any particular hybrid combination. No measure was made of the subsequent performance of the larvae on the artificial diets, but no anomalies were noted, except in the case of conspecific *Pi,* which had a 10–20% slower larval development rate.

2. *Male Response*

Male response was assessed by behavioral bioassays, first by passing air over calling females and into boxes containing males, and second by capturing males in traps baited with virgin females in a wind tunnel. Preliminary experiments were done by placing five to 10 males in plastic boxes (15 cm deep × 15 cm diam.). Air was then passed through a vacuum flask containing five virgin females and into the boxes containing the males at a rate of 1 lpm. Rapid wing vibration by the males while still walking on the substrate was taken as a positive response. Prior to the bioassays the females were kept on a 17-h photoperiod and were used between 2 h before and 2 h after lights-out to ensure that the females were calling. The males in groups of 5 or 10, 2 to 4 days old, were kept under continuous illumination and were used for bioassays of more than one type of female, an interval of at least 1 h being left between bioassays.

Table I. The percentage of successful matings between various combinations of female and male *Choristoneura* placed together as pairs in 8-oz glass jars with foliage

Combination ♀	Combination ♂	*n*	% females containing spermatophore	% mated females producing fertile eggs*	Av. no. of 2nd-instar larvae in hibernacula per female producing fertile eggs
Fu	*Fu*	20	85	100	186
Pi	*Pi*	20	65	38	133
Oc	*Oc*	2	100	50	210
Vi	*Vi*	23	83	47	90
Fu	*Pi*	20	90	61	93
Pi	*Fu*	20	85	76	97
Fu	*FuPi*	20	80	94	154
Fu	*PiFu*	19	74	86	105
Pi	*FuPi*	13	85	91	155
Pi	*PiFu*	15	93	93	147
FuPi	*Fu*	21	96	75	89
FuPi	*Pi*	22	59	38	38
PiFu	*Fu*	20	90	78	106
PiFu	*Pi*	20	35	71	48
Vi	*Fu*	11	54	67	103
Fu	*Vi*	8	88	86	178
Fu	*FuVi*	20	60	83	115
Fu	*ViFu*	20	65	92	146
Vi	*FuVi*	16	62	80	65
Vi	*ViFu*	15	73	54	92
FuVi	*Fu*	20	90	78	118
FuVi	*Vi*	18	72	100	75
ViFu	*Fu*	22	77	94	115
ViFu	*Vi*	20	65	85	60

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Oc* = *C. occidentalis*; *Vi* = *C. viridis*.

Female parent named first in hybrids.

*Number of females producing fertile eggs as a percentage of females containing a spermatophore.

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The results (Table II) confirm that *Fu* and *Pi* are not cross-stimulatory. It is also evident that *Vi* females did not produce significant amounts of pheromone before lights-out and that *Vi* males did not respond when kept under continuous illumination, but pheromone release and male response were very strong shortly after lights-off when males and females were kept on a light/dark cycle. The poor response of *Pi* males may also be due to an inappropriate light regime. The strong response by *Vi* males to *Pi* females (14 out of 20) is evidence that TDACET (a known sex attractant for male *Vi*) may be a part of the sex pheromone of *Pi*.

The evidence is that most male hybrids tend to respond to females of either parent species. The lack of response of *Vi* hybrids to *Vi* females is probably due to the lack of significant pheromone production by *Vi* females during the light period; assuming that *Pi* females produce TDACET, the fact that *Vi* hybrids responded to *Pi* females suggests that they would also respond to *Vi* females if the latter were producing pheromone.

The response of male *FuPi* and *PiFu* hybrids was further evaluated in a wind tunnel. The tunnel was 2.5 m long and 60 cm in cross-section with the roof and one side of plexiglass to permit illumination and observation. Two 3M Brand Sectar 1 traps, one baited with two virgin female *Fu* and the other with two virgin female *Pi*, were placed at the upwind end. Males were released at the downwind end. To avoid the possibility of a low response by *Pi* males in continuous illumination, males and females of both species and the male hybrids were maintained on a 17-h photoperiod and the experiment was begun shortly before lights-off, at which time the females of both species should be calling (Sanders 1971*b*). The following morning the catches were recorded and the experiment repeated. The position of the females was altered in each experiment to compensate for any bias the males might have for one side or other of the tunnel. Runs were also conducted with *Fu* and *Pi* males to ensure that the tests were valid. The experiments were conducted during the period 26 February to 8 March 1974. Males of only one species (or hybrid) were placed in the wind tunnel for each run to avoid problems of identification. The numbers involved varied each day, depending upon the numbers available. The minimum was 10 (*Pi*), the maximum was 91 (*FuPi*), but as far as was possible equal numbers of males were run with the *Fu* female on the left side of the tunnel, the *Pi* on the right, as were run with the *Fu* on the right and the *Pi* on the left.

Table II. The percentage of males of various *Choristoneura* species and their hybrids responding in laboratory bioassay to sex pheromone of the females of three species*

	Males responding to:					
	C. fumiferana ♀		*C. pinus* ♀		*C. viridis* ♀	
Male	Total number tested	% responding	Total number tested	% responding	Total number tested	% responding
Fu	45	69	25	0	10	0
Pi	30	0	100	33	50	4
Vi (before dark)	30	0	85	2	70	1
(after dark)	10	10	20	70	10	70
FuPi	130	28	45	38	0	0
PiFu	105	16	80	21	0	0
FuVi	50	20	20	30	25	0
ViFu	50	2	25	4	30	0
OcVi	15	33	10	0	10	0
ViOc	40	28	40	35	50	8

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Oc* = *C. occidentalis*; *Vi* = *C. viridis*.

*Males tested in groups of 5 or 10.

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Table III. Percentage of males captured in traps baited with virgin female *C. fumiferana* (*Fu*) or *C. pinus* (*Pi*) in wind tunnel

Males	Total number of males tested	Bait	
		2 female *Fu*	2 female *Pi*
Fu	341	37%	3%
Pi	20	0	10%
FuPi	155	11%	14%
PiFu	105	15%	18%

The results (Table III) confirm those in Table II, suggesting that hybrid males respond equally to females of both parent species, but they also allow the possibility that individual males may respond to one parent but not the other. Because it was not possible in these two experiments to determine if individual hybrid males would respond to both a *Fu* female and a *Pi* female, further bioassays were conducted in which individual hybrid males were tested against both parent females. As in the first experiment (Table II) males were held in plastic boxes (15 cm deep × 15 cm diam.) and air which had passed through a vacuum flask containing calling virgin females was blown in. Half the males were subjected to *Fu* pheromone, half to *Pi*. Those males responding were removed, placed in another container and bioassayed against the other female species 1 h later. The results (Table IV) indicate clearly that the majority of responsive males respond to both *Fu* and *Pi* females. The reason for the lack of response of the other males is not known. In all experiments involving pheromone response a considerable number of males fail to respond. This may be due to the physiological state of the insect, or, as is possible in this case, to an inherited inability to perceive either pheromone.

3. *Female Calling*

Sanders and Lucuik (1972) showed that 50% of *Fu* females start calling 3½-4½ h before lights-out at a temperature of 20°C under a 17-h photoperiod. In the field female *Pi* start calling 3–4 h later than *Fu* females (Sanders 1971*b*). Observations were therefore made on the effects of hybridization on calling behavior. One-day-old females reared under 17-h photoperiod were placed individually in 12-oz (340-ml) plastic containers, left for a further 24 h under 17-h photoperiod and then observed every 30 min to determine when each started calling (visible protrusion of the sex pheromone-producing gland at the tip of the abdomen). The percentage of females calling is shown in Table V. Except for *Or* and *Bi*, for which few females were available, all observations were repeated with different females, as they became available from rearings, on 2 or more days; the results (Table V) are the pooled data from all observations. No *Oc* females were available for this experiment, but of the species observed, *Pi*, *Vi*, and *Or* achieved 50% calling after lights-off, although in each

Table IV. Percentage of hybrid males responding to air passing over calling female *C. fumiferana* (*Fu*) or *C. pinus* (*Pi*)

Males	Number tested	Positive to *Fu* and *Pi*	Positive to *Fu* only	Positive to *Pi* only
FuPi	100	46%	13%	2%
PiFu	46	50%	11%	13%

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case females were apparently able to predict lights-off, since some individuals were visibly calling before lights-off. In *Bi* the onset of calling is much earlier; 50% of the females began calling 6 h before lights-off and all were calling 4 h before lights-off, possibly an adaptation to a montaine environment where evening temperatures are cool, necessitating activity earlier in the day. As previously reported, *Fu* achieved 50% calling by 3 h before lights-off. In the *Fu* × *Pi* reciprocal crosses there is evidence that the start of calling is influenced more by the male parent than by the female, the *PiFu* females following the *Fu* pattern, the *FuPi* the *Pi*. However, in the *Vi* × *Fu* reciprocal crosses the evidence is less precise; calling of the *FuVi* was closer to the *Vi* pattern, while six of 20 *ViFu* females started calling 3 h before lights-off, resembling the *Fu* pattern, but 50% calling was not achieved until after lights-out.

4. *Female Attraction in the Field*

The most effective method of determining which pheromones the hybrid females are releasing is to deploy traps in the field baited with the virgin females and to record the species of male caught. This was done with hybrid females in "wild" populations of *Fu* and *Pi* in Ontario, *Oc* in Oregon, and *Vi* and *Su* in California. The females were housed in small screen cages which were placed inside the traps. In Ontario 3M Brand XC-26 traps or SA21 reloadable traps were used; in Oregon Sectar 1 traps were used. Where possible the females were placed out in the field the second day after emergence, but occasionally females were 1 or 3 days old when placed out. Only one female was placed in each cage. The traps were located at a height of approximately 2 m with 7–10 m between traps. The experiments were carried out from 1970 to 1974. In each year females had to be placed out as they became available, and since resident male populations varied from day to day direct comparison between catches on different days was impossible. Therefore, at least five traps baited with "native" conspecific females were placed out with each series of hybrids, and the average catch of these five females was used as a standard. Traps were deployed in random sequence. They were checked after 2 days, when each trap was moved ahead to replace the next trap in the line so as to

Table V. The percentage of virgin females of various *Choristoneura* species and their hybrids calling at hourly intervals under a 17-h photoperiod, 50% calling denoted by box around number

Hours before or after lights-out	Percentage of females calling								
	Fu	*Pi*	*Vi*	*Or*	*Bi*	*FuPi*	*PiFu*	*FuVi*	*ViFu*
−8	0								
−7	0				20				
−6	0				[70]				
−5	37				80				
−4	40	5		10	100		25		10
−3	[67]	5		10	100		35	20	30
−2	77	25	6	10	100	10	[70]	13	35
−1	97	30	3	30	100	15	80	27	35
0	90	30	9	30	100	20	80	[53]	45
+1	100	[75]	49	40	100	[70]	100	73	[95]
+2	100	95	[97]	[70]	**100**	**85**	**100**	**87**	**95**
+3	100	100	100	100	100	100	100	100	100
n	30	20	35	10	10	20	20	15	20

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Vi* = *C. viridis*; *Or* = *C. orae*; *Bi* = *C. biennis*; *Oc* = *C. occidentalis*.
Female parent named first in hybrids.

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avoid some of the effect of trap location on the catches. Traps were collected after 4 days. The average catch of the empty check traps was subtracted in all cases, and the remaining figure was expressed as a percentage of the average catch of the conspecific female (minus the average of the empty check traps). All insects captured were examined and identified. In the case of *Fu* and *Pi* no problems in identification were encountered. The males of the two species are easily and reliably separated by color, even after being stuck in the adhesive for some time; male *Fu* are invariably grey, while male *Pi* are ocherous tawny. In western North America there is the danger of confusion since color polymorphism occurs in all species. However, each species does have some unique colormorphs and their occurrence in a trap is positive evidence for attraction of that species. The danger arises where it is possible for more than one species to occur in a trap. This problem was kept to a minimum by placing traps out in areas where only one of the species was known to occur.

The results (Tables VI–X) are expressed in two ways. First, the average catch for each combination is presented as a percentage of the catch by the conspecific females. This has only limited value, however, because particularly among the backcrosses, a situation could arise where some females are strongly attractive while others are unattractive and the average of 50% would be meaningless. The second method of presentation is to record the number of females showing no attraction and the number showing some attraction. Females with a zero catch after correction for the catches in the check traps are considered unattractive. Attractive females were further subdivided into those slightly attractive (catching 1–25% of the catch by the conspecific females) and those strongly attractive (catching more than 25% of the number caught by the conspecific females).

Catches of male *Fu* and male *Pi* are recorded in Table VI. To give some measure of actual numbers of males caught, the average catch by the 185 *Fu* females was 72.1 per female, while average catches by five *Fu* females on different days varied between 10.0 and 192.2 males per female. The average catch by *Pi* females of *Pi* males was 39.1 per female, varying on different days between a minimum of 8.0 and a maximum of 169.3 per *Pi* female.

As already reported (Sanders 1971*a*) male *Fu* were strongly attracted to female *Oc* and *Bi*, as well as to *Fu* females, while *Pi* males were strongly attracted only to *Pi* females, with slight attraction to *Vi* and *Or* females. Among the hybrids only the *Pi* × *Vi* reciprocal crosses were consistently unattractive to male *Fu*. Any combination including a *Fu* or an *Oc* as one of the parents showed some degree of strong attraction to *Fu* males (the one exception was an *OcPi* where only one female was available for testing). The sex of the *Fu* or *Oc* parent was not important. The reciprocal crosses were very similar both in average catch and in the percentage of attractive vs. unattractive. Fewer hybrid combinations were attractive to *Pi* males; the only strong attraction was to the *Pi* × *Vi* reciprocal crosses, and to some *PiFu*, although the reciprocal cross *FuPi* was completely unattractive. Attraction of *Pi* males to the *Oc* × *Vi* reciprocal crosses was never strong, but sufficient males were caught to indicate some affinity.

All the testing of backcrosses was done in 1974, including conspecific and F_1 hybrids for comparison (Table VII). In these experiments catches of *Fu* males by *Fu* females averaged 95.3 males per female (ranging from 34.8 to 192.2 for different days), while catches of *Pi* males by *Pi* females averaged 29.9 (range 13.0 to 55.8). The results can be summarized as follows:

(1) The higher the probability of a combination containing *Fu* genes, the higher the catch of *Fu* males (i.e., daughters of a *FuPi* male backcrossed to a *Fu* female (= *Fu* × *FuPi*) are more likely to be attractive to *Fu* males than those of a *Pi* × *FuPi*). The same applies in the trapping of the *Pi* males.

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(2) It seems to make little difference which parent is conspecific and which is a hybrid (i.e., the female offspring of a *PiFu* male backcrossed to a *Fu* female (*Fu* × *PiFu*) are similar in attractiveness to those of a *PiFu* × *Fu*, etc.).

(3) As with F_1 hybrids, attraction of *Fu* males is more prevalent than attraction of *Pi* males. Thus, in the backcrosses most attractive to *Fu* males, 65% or more of the females were strongly attractive. In those backcrosses most attractive to *Pi* males, only 25% were strongly attractive to *Pi* males and even in the same cross 17% were strongly attractive to *Fu* males.

The results of trapping *Oc* males and *Vi* males are shown in Table VIII. The average catch of *Oc* males by *Oc* females was 33.9 per female (ranging from 16.6 to 51.2) and of *Vi* males by *Vi* females 18.9 per female (range 14.5 to 25.2). Male *Oc* were most strongly attracted to female *Oc* and female *Fu*, which is not surprising since the male *Fu* are strongly attracted to female *Oc*, but *Oc* males were also attracted to some *Pi* and *Su* females, species which did not attract male *Fu*. Male *Vi* were also attracted to some females of all species except *Su*. Data for hybrid and backcross attraction of *Oc* and *Vi* males are sparse, but the available evidence suggests a trend similar to that found for *Fu* males, i.e., the combinations in which TDAL-producing genes prevail were highly attractive to male *Oc*, while combinations with TDACET-producing genes predominating were attractive to *Vi* males.

Table VI. Attractiveness of virgin female *Choristoneura* of several species and their F_1 hybrids to male *C. fumiferana* and male *C. pinus*, Sault Ste. Marie, Ont., 1970–1974, showing the average catch per female (as percentage of catch by the conspecific female) and the percentages of the females attractive and non-attractive

	vs. male *C. fumiferana*					vs. male *C. pinus*				
Female	*n*	Av. catch ± 1 S.E. (as % of catch by attractive *Fu* females)	Non-attractive (%)	Attractive* 1–25%	Attractive* > 25%	*n*	Av. catch ± 1 S.E. (as % of catch by attractive *Pi* females)	Non-attractive (%)	Attractive* 1–25%	Attractive* > 25%
Fu	185	96.3± 3.2	2	3	95	60	1.8±0.2	95	5	0
Pi	52	0	100	0	0	62	99.8±6.8	1	2	95
Oc	29	53.3±12.5	3	45	52	5	0	100	0	0
Vi	23	0	100	0	0	9	1.4±1.0	78	25	0
Bi	15	57.7±11.2	0	27	73	–	–			
Or	15	0	100	0	0	3	18.7±3.5	0	100	0
Su	11	0	100	0	0	11	0	100	0	0
FuPi	85	34.9± 3.9	22	28	50	46	0	100	0	0
PiFu	80	46.4± 4.5	18	25	57	31	12.0±6.2	71	19	10
FuOc	14	72.6±19.7	7	14	79	11	3.6±2.9	91	9	0
OcFu	5	72.5±27.7	20	0	80	1	0	100	0	0
FuVi	13	38.1±15.6	25	42	33	2	0	100	0	0
ViFu	31	51.0± 8.9	26	13	61	5	0	100	0	0
PiVi	12	0	100	0	0	7	37.1±8.7	0	29	71
ViPi	15	0	100	0	0	8	26.1±7.4	12	38	50
PiOc	15	41.5± 7.7	27	6	66	10	0	100	0	0
OcPi	1	0	100	0	0	1	0	100	0	0
OcVi	28	24.3± 5.0	25	46	29	21	3.1±1.6	91	9	0
ViOc	31	26.2± 4.7	29	30	41	22	3.3±1.2	90	10	0

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Oc* = *C. occidentalis*; *Vi* = *C. viridis*; *Bi* = *C. biennis*; *Or* = *C. orae*; *Su* = *C. subretiniana*.

*The attractive females are subdivided into those slightly attractive (catching less than 25% of the conspecific catch) and those strongly attractive (catching more than 25%).

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The trapping of *Su* males was limited to one experiment (Table IX). Traps baited with the available females of the various species were deployed in random sequence and left for 7 days. The average catch by the 7 *Su* females was 115.0±20.2 males per female. The *Su* males were strongly attracted only to their own females but there was some evidence of attraction to *Pi* and *Vi,* the TDACET-producing species, and also to *Oc,* although again numbers were low.

In 1974 large numbers of *Fu* males were present in a stand where trapping of *Pi* males was being carried out. The male *Fu* presumably originated from a very extensive infestation further north, where development was slightly slower. As a result it was possible to determine for individual females whether they were attractive to either *Pi* or *Fu* males, to both, or to neither. Again, traps were baited with 1-day-old females, deployed in random sequence in lines, 10 m apart. They were checked and moved ahead to replace the next trap in line after 2 days and were collected after 4 days. Since females had to be deployed as they emerged from rearings, experiments were conducted over several days. Each sequence included five *Pi* and five *Fu* females. Catches of male *Fu* by *Fu* females averaged 136.4 males per female (range 92.8 to 189.4 per day) while *Pi* catches by *Pi* females averaged 29.9 (range 13.0 to 55.8 on different days). The first notable feature of the results (Table X) is that no individual female was attractive to both males. The second is that a significant number of individuals of both hybrids and all four backcrosses were unattractive to both parent species, although unattractive females were rare among the parent species. This, coupled with the fact that the number of males caught and the proportion of unattractive females were similar in the reciprocal crosses, suggests that unattractiveness was due to inappropriate pheromones and not merely to lack of vigor.

Table VII. Attractiveness of virgin female *C. fumiferana, C. pinus,* and their F_1 hybrids and backcrosses to male *C. fumiferana,* and male *C. pinus,* Sault Ste. Marie, Ont., 1974, showing the average catch per female (as percentage of the catch by the conspecific females) and the percentage of the females attractive and non-attractive

	vs. male *C. fumiferana*					vs. male *C. pinus*				
Female	*n*	Av. catch ± 1 S.E. (as % of catch by attractive *Fu* females)	Non-attractive (%)	Attractive*		*n*	Av. catch ± 1 S.E. (as % of catch by attractive *Pi* females)	Non-attractive (%)	Attractive*	
				1–25%	> 25%				1–25%	> 25%
Fu	71	93.8± 4.9	4	4	92	30	0	100	0	0
Pi	29	0	100	0	0	29	100±9.8	0	3	97
FuPi	65	43.1± 4.6	11	34	55	32	0	100	0	0
PiFu	54	43.0± 5.7	24	22	54	23	14.0±8.5	74	17	9
Fu×FuPi	47	91.1±10.6	6	21	73	21	0.3±0.3	95	5	0
FuPi×Fu	8	108.5±20.6	0	0	100	0	–			
Fu×PiFu	43	63.6± 8.3	12	23	65	18	0	100	0	0
PiFu×Fu	25	77.4± 9.2	12	4	84	0	–			
Pi×FuPi	69	12.7± 3.2	64	19	17	32	25.3±8.3	56	19	25
FuPi×Pi	3	0	100	0	0	0	–			
Pi×PiFu	61	8.1± 2.4	66	23	11	31	23.1±8.5	52	26	22
PiFu×Pi	10	11.7±10.5	70	20	10	0	–			

Fu = *C. fumiferana*; *Pi* = *C. pinus*.

*See footnote Table VI.

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Table VIII. Attractiveness of virgin female *Choristoneura* to male *C. occidentalis* and male *C. viridis* showing the average catch per female (as percentage of the catch by the conspecific females) and the percentage of the females attractive and non-attractive

	vs. male *C. occidentalis*					vs. male *C. viridis*				
Female	*n*	Av. catch ± 1 S.E. (as % of catch by attractive *Oc* females)	Non-attractive (%)	Attractive* 1–25%	Attractive* > 25%	*n*	Av. catch ± 1 S.E. (as % of catch by attractive *Vi* females)	Non-attractive (%)	Attractive* 1–25%	Attractive* > 25%
Fu	11	90.8±22.7	0	9	91	9	18.3± 3.4	56	11	33
Pi	4	32.3±11.5	0	50	50	5	22.4± 7.5	20	40	40
Oc	10	100±20.5	0	10	90	9	8.0± 2.9	50	38	12
Vi	13	4.6± 1.7	62	38	0	18	89.1±13.8	11	6	83
Su	6	37.8±21.6	16	50	34	4	0	100	0	0
ViOc	4	117.5±23.0	0	0	100	0	0	–		
Vi×*ViOc*	5	2.8± 1.4	40	60	0	5	67.4±67.4	80	0	20
FuVi	0	–				7	0	100	0	0
Vi×*FuVi*	4	1.5± 1.0	50	50	0	4	0	100	0	0
FuVi×*Vi*	0					14	22.4±14.4	71	7	22
Fu×*FuVi*	3	92.3±33.0	0	0	100	1	0	100	0	0
FuVi×*Fu*	1	88	0	0	100	3	0	100	0	0
Vi×*ViFu*	0	–				1	0	100	0	0
ViFu×*Vi*	0	–				5	0	100	0	0
Fu×*ViFu*	3	51.3±32.0	0	33	67	11	1.5± 1.1	82	18	0
ViFu×*Fu*	3	73.3±21.4	0	0	100	3	0	100	0	0

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Oc* = *C. occidentalis*; *Vi* = *C. viridis*; *Su* = *C. subretiniana*.

*The attractive females are subdivided into those slightly attractive (catching less than 25% of the conspecific catch) and those strongly attractive (catching more than 25%).

5. *Pheromone Interaction*

In earlier studies it had been noted that when traps baited with TDAL also contained either TDACET or TDOL, catches of male *Fu* were much reduced (Sanders *et al.* 1972; Sanders 1976).

Experiments conducted in 1973 demonstrated that catches of male *Fu* in traps baited with female *Fu* were reduced by the presence of TDACET or TDOL (Sanders 1976). Since the calling behavior of the females was not affected by the presence of

Table IX. Attractiveness of *Choristoneura* females to male *C. subretiniana* showing the average catch per female (as percentage of the catch by a *C. subretiniana* female) and the percentage of the females attractive and non-attractive

Females	*n*	Av. catch ± 1 S.E.	Non-attractive (%)	Attractive* Slightly	Attractive* Strongly
Fu	5	0	100	0	0
Pi	5	3.0± 1.9	60	40	0
Vi	6	4.3± 1.8	67	33	0
Oc	7	2.4± 1.9	86	14	0
Su	7	115.0±20.2	0	0	100

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Vi* = *C. viridis*; *Oc* = *C. occidentalis*; *Su* = *C. subretiniana*.

*The attractive females are subdivided into those slightly attractive (catching less than 25% of the conspecific catch) and those strongly attractive (catching more than 25%).

Table X. Percentage of virgin female *Choristoneura* attractive to male *C. fumiferana* and *C. pinus* in an area of sympatric synchronous populations, Sault Ste. Marie, Ont., 1974

Females	*n*	Attractive to *Fu* males	Attractive to *Pi* males	Attractive to both *Fu* and *Pi*	Non-attractive to both *Fu* and *Pi*
Fu	30	97	0	0	3
Pi	29	0	100	0	0
FuPi	32	78	0	0	22
PiFu	23	57	26	0	17
Fu×*FuPi*	20	85	5	0	10
Fu×*PiFu*	18	89	0	0	11
Pi×*FuPi*	32	22	44	0	34
Pi×*PiFu*	31	23	48	0	29

Fu = *C. fumiferana*; *Pi* = *C. pinus*.

either chemical, it is assumed that it is male response that was affected, and not the production of the pheromone by the calling female. In contrast to the situation in *Fu*, catches of *Oc* males were not reduced when TDACET was present in the trap with an *Oc* female (Daterman, unpub. data). Since we presume that *Fu, Oc,* and *Bi* females produce TDAL whereas *Pi* and *Vi* females produce TDACET, the question arises of what effect hybridization will have where one parent produces TDAL, the other TDACET. The female may produce the pheromones of both parent species, which could result in a blend of chemicals which is not attractive to males of either species. The consequences of such a possibility were investigated by placing pairs of females, housed in separate cages, in the same traps. These were then deployed in Ontario, Oregon, and northern California, with the experimental design as in the previous experiments. The results (Table XI) show that catches of *Fu* and *Pi* males were reduced when females of both species were present in the same trap. Catches

Table XI. Catches of conspecific males by virgin female *Choristoneura* (female 1) when placed alone in traps, and when in the presence of an alien female (female 2) in the same trap. Where differences are significant, inhibition of the males is presumed to have occurred

		Female 1 alone		Female 1 + Female 2		
Female 1	Female 2	*n*	Av. catch ± 1 S.E.	*n*	Av. catch ± 1 S.E.	*t*
Fu	*Pi*	10	36.7± 6.5	9	10.1± 3.5	3.50**
Fu	*Pi*	10	28.9± 3.3	9	7.0± 2.5	2.29**
Fu	*Pi*	8	31.9± 8.9	9	20.8± 7.1	0.98 ns
Fu	*Pi*	10	38.7±12.1	10	20.7±10.4	1.13 ns
Fu	*Vi*	5	81.6±14.5	5	44.0±16.8	1.69*
Pi	*Fu*	3	51.0±21.7	5	12.8± 5.8	2.17**
Oc	*Vi*	5	51.2±16.4	4	43.8±18.5	0.30 ns
Oc	*Vi*	5	16.6± 4.9	3	17.7± 5.2	0.14 ns
Oc	*Pi*	5	51.2±16.4	2	37.0±12.3	0.46 ns
Oc	*Fu*	5	16.6± 4.9	3	10.7± 1.3	0.90 ns
Oc	*Su*	5	51.2±16.4	4	34.0± 5.2	0.80 ns
Vi	*Oc*	10	22.5± 5.1	6	7.5± 5.6	1.94*
Vi	*Oc*	4	14.5± 2.7	4	21.2± 6.7	0.93 ns
Su	*Vi*	7	115.0±20.2	3	22.3± 9.6	2.85**
Su	*Oc*	7	115.0±20.2	2	33.0±10.0	2.17**

Fu = *C. fumiferana*; *Pi* = *C. pinus*; *Vi* = *C. viridis*; *Oc* = *C. occidentalis*; *Su* = *C. subretiniana*.

*,**Difference significant at $P = 0.1$ and $P = 0.05$ respectively.

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of *Fu* males were also reduced by the presence of a *Vi* female. However, there was no significant reduction in catches by *Oc* females when in the presence of a *Vi, Pi,* or *Su* female, nor was there a reduction in catches of *Vi* males by female *Vi* in the presence of an *Oc* female. Catches of *Su* males by *Su* females were reduced by the presence of either a *Vi* or an *Oc* female.

Discussion and Summary

The effects of hybridization among the various species of *Choristoneura* on sterility and vigor of the progeny have not been thoroughly investigated. Smith (1953) found no exceptional sterility in the eggs of *Fu* × *Pi* hybrids although some hybrid females showed more sterility than the conspecifics. Campbell (1961) found some slight sterility in the eggs of *Fu, Pi,* and *Oc* hybrids. Harvey (pers. comm.) has found lower mating success in *Fu* × *Oc* hybrids than in conspecific matings, and has further pointed out that there are differences in egg weight, fecundity, insect size, and behavior of hybrids, all of which may contribute to variations in survival and vigor of the progeny. However, there are no major differences evident among the various hybrid combinations (Table I). It is therefore assumed in the following discussion that the performance of the insects is a true indication of their pheromonal production and response, and that hybrid sterility and loss of vigor are not significant factors.

Most individual hybrid males of the reciprocal *Fu* × *Pi* crosses responded to the pheromones produced by the females of both parent species (Table III), which are assumed to be TDAL and TDACET, even though *Fu* and *Pi* males are attracted only to females of their own species (Table XI). Single pheromone receptors on the antennae of male *Fu* respond to both TDAL and TDACET (W. D. Seabrook, pers. comm.). Evidently a male *Fu* can differentiate between the two chemicals, but possibly hybrid males no longer make the differentiation.

The effect of hybridization on the females was evaluated in two ways: first by observing calling behavior, and second by determining, under field conditions, the attractiveness of the hybrid females to males of the various species. The start of calling shows considerable variation among the different species. *Bi* females start 6 or 7 h before dusk, *Fu* 3–5 h before dusk. *Pi* and *Or* start shortly before dusk, but achieve maximum only after dusk, while *Vi* call only after dusk. Unfortunately, recording the start of calling is somewhat subjective, and thus it cannot be used as a definitive measure of the effects of hybridization. However, it is evident that the start of calling in the hybrids is intermediate between that of the two parent species, as was found with hybrid female tussock moths (Grant *et al.* 1975), with indication in at least the *Fu* × *Pi* reciprocal crosses that they tend to follow the pattern of the male parent species more closely.

The field trapping data provide a more precise picture of the effects of hybridization, since the numbers of males captured by females in competition with other females show clearly not only which species of male responds, but also how potent the female is relative to other females. However, before discussing the effects of hybridization, it is necessary to clarify the relationships among the species involved.

Pheromones of the Parent Species

The pheromone communication system of *Fu* contains TDAL plus a low proportion of CDAL (Sanders and Weatherston 1976). *Oc* and *Bi* are both attracted to commercial TDAL (which contains traces of CDAL), and since in all interactions tested among the three species they are mutually attractive (Tables VI and VIII and Sanders *et al.* 1972) it is assumed that the pheromones of *Oc* and *Bi* are also predominantly TDAL.

As well as hybridizing readily under the restrictions of small cages in the laboratory, *Fu* and *Oc* have been found to hybridize in a large field cage enclosing a whole tree (Harvey and Stehr, pers. comm.). Presumably they would hybridize and produce viable offspring in the field were it not for their geographical separation. This raises the question of whether *Fu* and *Oc* should be considered separate species or subspecies, a question that can be answered only by intensive examination of the differences explored by Harvey (1967) and Harvey and Stehr (1967), and possible differences in the minor components of the pheromone systems as indicated here. *Fu* and *Oc* males show different response to their conspecific females when in the presence of alien females or non-pheromone chemicals. Catches of male *Fu* by *Fu* females are reduced in the presence of TDACET or TDOL (Sanders 1976). Catches of *Fu* males are also reduced when the *Fu* female is in the presence of a *Pi* or *Vi* female (Table XI), both of which are assumed to produce TDACET. However, catches of *Oc* males are not reduced by the presence of *Pi* or *Vi* females (Table XI), or by the presence of TDACET (Daterman, unpub. data).

Not only are catches of *Oc* males not reduced by the presence of other females, they are slightly attracted by them (Table VIII). *Vi* males are also attracted by all females except *Su*. Since *Oc* males are attracted to TDAL and *Vi* males to TDACET, it might be concluded that all of the females tested produce some TDAL and some TDACET. However, since catches of male *Fu* are reduced by the presence of TDACET in concentrations down to 1:1000 (TDACET:TDAL) (Sanders 1976) this seems unlikely. *Vi* males have shown slight attraction to TDAL (Sanders *et al.* 1974) but it is questionable if that could account for the numbers caught here (Table VIII). The only other alternative is that the females of all the species tested have some minor component of their pheromone system in common.

As mentioned above, there is some question as to whether *Fu* and *Oc* should have specific or subspecific status, and since *Bi* females strongly attract *Fu* males, possibly *Bi* should be considered as a subspecies also. However, the status of the other species is supported by the evidence of their pheromone responses. The fact that *Vi* and *Oc* larvae have been found together in the same field collections indicates that they are partially sympatric. However, they show only slight interspecific attraction. *Or* and *Bi* may be partially sympatric. Although the data are limited, it is probable that the integrity of the two is maintained by different major components of their pheromone systems. The range of *Su* is only partly known; it is certainly partially sympatric with *Vi,* and possibly with *Oc*. There is, however, only a slight response by *Su* males to *Oc* and *Vi* females, and the lack of attraction of *Vi* males to *Su* females and the strong reduction in catches of *Su* males by *Su* females in the presence of *Vi* and *Oc* females certainly suggest separate specific status.

Effects of Hybridization

Since the evidence indicates that both *Fu* and *Oc* utilize TDAL, it is no surprise to find that females of their reciprocal hybrids are strongly attractive to male *Fu*. Similarly, reciprocal *Vi* × *Pi* hybrids are strongly attractive to male *Pi,* and this suggests again the presence of a common factor, presumably TDACET, in the pheromone of these two species. In hybrids involving one parent from the TDAL group (*Fu* or *Oc*) and one from the TDACET group (*Vi* or *Pi*) the picture is more complex.

Any F_1 hybrid combination involving *Fu* or *Oc* as one parent shows some attraction to male *Fu* (with the exception of *OcPi,* Table VI, where only one female was available for testing). Only one F_1 hybrid (*ViOc*) was available for testing against *Oc,* but it too was strongly attractive. Since these hybrids attracted *Fu* males, regardless of which parent was the producer of TDAL, it is concluded that inheritance is via either parent and therefore not exclusively on the sex chromosome. However, in the *Fu* × *Pi*

reciprocal crosses those females with *Pi* as the female parent showed stronger attraction to male *Pi* than those females with *Fu* as the female parent, which suggests that inheritance is associated to some extent with sex, a situation similar to that found by Grant *et al.* (1975) in hybrid tussock moths.

In hybrid combinations where some individual females attracted one species of male and others attracted another, it was evident that no individual female was attractive to males of both species. For example, where *Fu* males and *Pi* males were flying contemporaneously, some *PiFu* hybrid females attracted male *Pi* and others attracted male *Fu*, but none attracted both (Table X).

Possible Explanations for Responses to Hybrid Females

1. *Genetic*

In the absence of chemical analysis, identification of phenotypes of hybrid females must rest on their attractiveness to males. A degree of uncertainty is thereby introduced into some of the observed ratios, as it is not possible to determine if the relatively large proportions of totally unattractive females in the F_1 and backcross to *Pi* (Table X) are attributable to the failure of the females to produce pheromone or to the failure of the males to detect it. Because of this uncertainty and because of the relatively low numbers of females available for testing, it is not possible to establish the exact ratios. However, the accumulation of more data is likely to be a slow process. The occurrence of relatively high density sympatric, synchronous populations of *Fu* and *Pi* was unusual, and may not be repeated for many years. The following assessment is therefore of a preliminary nature and reviews the mechanisms that may be involved and the direction that should be taken by further genetic testing.

Since females from the F_1 crosses showed higher attraction to males of the TDAL-producing species than of the TDACET-producing species, it can be argued that the mechanism(s) regulating the production of TDAL is dominant with respect to that regulating the production of TDACET. This possibility is supported by the catches of the female *FuPi* and female *Fu* × *FuPi* (or *Fu* × *PiFu*), which were attractive to male *Fu* but not to male *Pi* (Tables VII, X). Females from backcrosses to the recessive type, i.e., *Pi* × *FuPi* or *Pi* × *PiFu*, should attract *Fu* and *Pi* males in an approximately 1:1 ratio which is not inconsistent with the results. However, the fact that some *PiFu* females caught significant numbers of *Pi* males is at odds with this interpretation of simple autosomal dominance, which would imply that no *PiFu* females should be attractive to *Pi* males.

Though autosomally identical with *FuPi*, *PiFu* females carry a single paternally derived *Fu* X chromosome. The appearance of two classes therefore suggests the segregation of alleles at an X-linked locus. That this expression is not completely sex-linked is shown by the *Fu* × *Pi* crosses: though carrying a single *Pi* X, they attracted only *Fu* males. Again, the low numbers involved do not permit exact determination of ratios, but it is possible that the exceptional ratios observed in *PiFu* can be attributed to the epistatic interaction of at least one autosomal and one X-linked locus.

Such sex-controlled and sex-limited epistatic interaction may not be uncommon in Lepidoptera. Sex-controlled inheritance has been described by Stehr (1959) for hemolymph color in *Choristoneura,* although in that case phenotypes were not limited to one sex but appeared in different ratios in both sexes. Numerous other examples of apparently sex-limited inheritance have been described in Lepidoptera (Robinson 1971, pp. 51–58) although in many cases they have not yet been thoroughly analyzed.

2. *Pheromone Interaction*

The fact that no individual hybrid female attracted males of both parent species suggests that individual females produce the pheromone of one or other of the parent

species, but never both. However, it is possible that they do produce both chemicals, but that the resulting blend is not attractive to males of either species, just as happens presumably when females of different species are placed in the same trap. This possibility is supported by the fact that at least some males inherit the ability to detect and respond to the pheromone of both parents. A *Fu* × *Pi* hybrid female would then be producing TDAL and TDACET simultaneously, which could explain the apparent lack of attraction or low attraction of many of the hybrid females. This interpretation is also supported by the fact that catches of male *Oc* (which were not reduced by the presence of TDACET) were higher in traps baited with hybrid females of the *Oc* × *Vi* and *Fu* × *Vi* crosses than were catches of male *Fu* (which are reduced by the presence of TDACET).

Also it is quite possible that in addition to the main chemical components of the pheromones, there are other chemicals present in minor amounts. This could lead to a variety of blends in the hybrid females, and would make interpretation of the results extremely difficult.

3. *Competition for Chemical Precursor*

Yet another alternative is that although individual hybrid females inherit the ability to produce the pheromones of both parents, there may be competition between the genes for chemical substrate. Both TDAL and TDACET most probably are derived from TDOL, which has been found in the abdominal tips of female *Fu* (Weatherston and Maclean 1974). If conversion to TDAL is achieved more readily than to TDACET, then it is possible that a hybrid with both sets of genes will end up with a preponderance of TDAL, thus attracting male *Fu* and *Oc* but with insufficient TDACET to attract male *Pi* or *Vi* or to reduce catches of male *Fu*.

None of the theories discussed above explains the results adequately. Nevertheless, there is no reason to suppose that one operates to the exclusion of the others. In such a complex chemical, physiological, and behavioral system as is dealt with here there may well be several mechanisms in operation that at times operate separately and at times operate together. A better appreciation of the true nature of the system can be obtained only by further detailed chemical analysis of the pheromones of individual hybrid females and by further genetic testing.

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