eter, 10 μm). Ethacrynic acid was then injected intravenously. A constant infusion of isotonic saline was maintained to replace urinary losses of salt and water. Approximately 10 minutes later, another sample of endolymph was obtained from the same ear; approximately 45 minutes after this, perilymph and endolymph were obtained from the contralateral ear. All pipettes were immediately sealed with mineral oil after collection of fluid. Deviations from this protocol were made when one ear was infected (dog No. 6) or when the basilar membrane was damaged during removal of the round window (dog No. 4).

The pipettes were made by pulling Pyrex tubing (inside diameter, 0.5 mm; outside diameter, 1.0 mm) to a tip diameter of 1 μm in a Narishige pipette puller. These pipettes were sharpened to a beveled tip of 10 or 20 μm with an air-driven grindstone, as described by Vurek *et al.* (*4*). After the pipette was partially filled with mineral oil, it was mounted on a Brinkman micromanipulator. Approximately 1 to 2 μl of fluid was obtained for analyses. Sodium, potassium, and osmolality were determined as described previously (*5*).

The control values of sodium and potassium in perilymph and endolymph of the dog (Table 1) are similar to those reported for other species (*6*). The mean control concentrations of sodium and potassium in perilymph in six dogs was 147 and 5.7 milliequivalents per liter (meq/liter), respectively. The mean concentration of sodium and potassium in endolymph was 5.9 and 145 meq/liter, respectively. The osmolalities of cochlear fluids were hypertonic as compared to plasma. The mean plasma osmolalities in the dogs studied was 286 milliosmols per kilogram of H_2O, with a range of 283 to 290.

After the administration of ethacrynic acid, at doses of 1 to 5 mg per kilogram of body weight, the mean concentrations of sodium and potassium in endolymph was 146 and 21.2 meq/liter, respectively, with no change in the osmolality. There was no change in the composition of perilymph after administration of ethacrynic acid (Table 1).

It is apparent from this study that doses of ethacrynic acid used in clinical medicine (1 mg per kilogram of body weight) cause changes in the composition of endolymph. Within 10 minutes of an intravenous administration, there is a profound change in the composition of endolymph which persists for at least 45 minutes (Table 1). Wilson and Juhn (*7*) reported an increase in the concentration of potassium in perilymph from the guinea pig after 48 hours of very large doses of ethacrynic acid. The present findings, however, demonstrate that there is no acute change in the composition of perilymph at a time when there are marked changes in endolymph. Perhaps the cochlear duct and the scala tympani are independent compartments with little ionic exchange between them, although the similarities of the respective osmolalities suggest water may equilibrate between the two compartments. The slight hypertonicity of cochlear fluids is surprising but is consistent with the data of Aldred, Hallpike, and Ledoux (*8*), who found the osmotic pressure of perilymph and endolymph to be equivalent to a 1 percent solution of sodium chloride.

It appears that sodium, potassium, and their anions do not account for the measured osmolality, although it is noteworthy that after administration of ethacrynic acid the unidentified solutes account for less than 10 milliosmols per kilogram of H_2O in endolymph.

Ethacrynic acid may exert its effect either by altering the permeability characteristics of the membranes surrounding the cochlear duct and allowing dissipation of the concentration gradients of sodium and potassium, or the drug may interfere with a specific ion transport system, as it does in other biological systems.

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Sex Pheromone Specificity and Taxonomy of Budworm Moths (Choristoneura)

Abstract. *Of six closely related species of budworm moths, three share the same or similar sex pheromones. Two of the others share another sex pheromone, but are geographically isolated. The sixth species, which is the most morphologically distinct, shows only slight affinity to the others.*

The genus *Choristoneura* of the lepidopterous family Tortricidae includes a group of coniferous defoliators which are of considerable economic importance in both western and eastern North America. Six of the presently recognized species were included in the single species *C. fumiferana* (Clem.) until 1953 when Freeman (*1*) described *C. pinus* Free., the jack-pine budworm, as a distinct species, a view supported by subsequent field observations (*2*). In 1967, *C. fumiferana* was further subdivided (*3*). The name *C. fumiferana* was restricted to the eastern spruce budworm of the boreal forests and the western populations were divided into four new species. These were *C. occidentalis* Free., the western 1-year-cycle budworm, ranging from British Columbia to New Mexico; *C. biennis* Free., the western 2-year-cycle budworm, of the mountains of British Columbia and Alberta with adults appearing in the even numbered years only; *C. orae* Free., the coastal budworm, from the coast of British Columbia; and *C. viridis* Free., the green budworm, from Oregon and California. Separation was based largely on differences in the frequencies of polymorphic traits as well as on differences in physiology, geographic range, and host preference. Reliable distinguishing characters could not be found in the male or female genitalia, and indeed the species will hybridize in the laboratory, although mating is not indiscriminate.

When these species were described, the importance of sex pheromones in mating behavior was not ap-

Table 1. Response of males of six species of *Choristoneura* to female sex pheromones in laboratory bioassays.

Females	Males responding (%)					
	C. fumiferana	*C. occidentalis*	*C. biennis*	*C. orae*	*C. viridis*	*C. pinus*
C. fumiferana	53	42	60	0	0	0
C. occidentalis	25	40				
C. biennis	0		5			
C. orae	2			36	0	20
C. viridis	0			0	0 (24)*	16
C. pinus	0	0		30	0	19

* Response to crushed tip of female abdomen.

preciated. Subsequently Roelofs and Comeau (*4*) have demonstrated the value of sex pheromone specificity in taxonomic studies, and I have previously reported the specificity of the sex pheromones of the two sympatric eastern species, *C. fumiferana* and *C. pinus* (*5*). I now report studies on the relationships among the sex pheromones of the other four species.

Collections of larvae and pupae of the four western species and *C. pinus* were obtained during June and July 1970 with the cooperation of personnel from the USDA Forest Service and the Canadian Forestry Service. Larvae were reared to maturity on their native host plant where available, or on eastern white spruce and balsam fir. After pupation the insects were sexed; adult males were kept for bioassays, females for the collection of pheromone and for field trials of their attractancy. Adult *C. fumiferana* were available throughout the experiments from field collections and laboratory-reared stock.

Solutions containing the sex pheromones were obtained by leaving groups of females in 1000-ml flasks for one or more nights and then rinsing out the flasks with ether (previous experiments with *C. fumiferana* had shown that extracts obtained by the more conventional means of cutting off the tips of the females' abdomens were biologically inactive). The resulting washes were made up to a concentration of one female-night per milliliter; that is, a wash from 100 females left for two nights would be made up to 200 ml. Bioassays were conducted by expelling air from a medicine dropper that had previously been used to suck up approximately 0.5 ml of the pheromone solution into plastic boxes containing males. Buzzing by the males (circling on the substrate with rapidly beating wings) was considered a positive response (*5*).

The results (Table 1) show that both *C. occidentalis* and *C. biennis* males responded to *C. fumiferana* washes whereas *C. fumiferana* males responded to *C. occidentalis* washes, though at a lower level than their response to washes from *C. fumiferana* females. Inexplicably, washes from *C. biennis* females were not biologically active. Neither *C. orae* nor *C. viridis* males responded to *C. fumiferana* washes; however, *C. orae* and *C. pinus* males responded equally well to washes of their own or each others' females. *C. viridis* washes produced no response from *C. viridis* males, nor from *C. fumiferana* or *C. orae* males. However, they did evoke a response from *C. pinus* males, and *C. viridis* males did respond when presented with the tip of the abdomen of a female *C. viridis* crushed on filter paper.

These results were supplemented by field experiments in which virgin females were used to trap males in outbreaks of the two eastern species (*C. fumiferana* and *C. pinus*) to determine their attractancy to the native males. Single females were housed in screen cages placed in the center of plywood boards 1 foot square, coated with Stickem Special (Michel and Pelton Co., Emeryville, California) which trapped the attracted males. In each experiment the numbers of traps baited with test females or with native females were equal. Control traps containing no females were set up in each experiment, and the number of males caught on the controls was deducted from the numbers on the baited traps. The traps were left for three or four nights in each experiment. Variations in the density of the male populations led to differences in the number of males available for trapping in each experiment. The catches have, therefore, been standardized by expressing them as percentages of the number of males caught by the native females in each experiment (Table 2).

The results indicate that *C. fumiferana, C. occidentalis,* and *C. biennis* share the same or similar sex pheromones, whereas *C. pinus* and *C. orae* share a pheromone distinct from the first group. The laboratory tests with *C. viridis* were inconclusive, but suggest some affinity between *C. viridis* and *C. pinus.* However, in the field trials *C. viridis* females competed only poorly with *C. pinus* females, indicating that the affinity is not strong. This, coupled with the distinctive coloration of *C. viridis* larvae and adults, suggests that the relationship between *C. pinus* and *C. viridis* is not as close as the relationships among the other species.

Although strong interspecific attractancy was demonstrated within the two groups, in no case did the attractancy of the alien females equal that of the native females. This suggests that there are subtle differences in the pheromones themselves or in the manner of their release. Observations on the females showed that in all species, calling (extrusion of the sex pheromone gland)

Table 2. Numbers of male *C. fumiferana* and *C. pinus* field-trapped by virgin females of six species of *Choristoneura*. The total number of traps involved (each containing one female) and the total number of males caught are included to indicate the reliability of the data. The relative catch is expressed as the percentage of the numbers caught by *C. fumiferana* and *C. pinus* females, respectively.

Females	*C. fumiferana* males			*C. pinus* males		
	Relative catches	Traps (No.)	Total caught (No.)	Relative catches	Traps (No.)	Total caught (No.)
C. fumiferana	100	61	1163	1	3	10
C. occidentalis	53	17	181			
C. biennis	64	18	293			
C. orae	0	3	3	18	3	95
C. viridis	0	6	0	4	4	15
C. pinus				100	7	895
Controls (no female)		21	13		7	7

began before or shortly after dusk, which coincides with the peak activity of both *C. fumiferana* and *C. pinus* males (*5*). Therefore, the timing of the sex pheromone release is unlikely to be an important factor. Possibly the actual quantities of pheromone released by the females differ with the species, but it is also possible that secondary chemicals are involved that increase the specificity of the sex pheromones of each species as has been suggested by Roelofs and Tette (*6*).

The apparent affinity between *C. orae* and *C. pinus* is surprising, for *C. orae* is more similar in polymorphic characters to the group of *C. fumiferana, C. occidentalis,* and *C. biennis.* Furthermore, *C. orae* feeds on spruce and fir, whereas *C. pinus* feeds on pine, a dichotomy that has been accepted as a natural taxonomic division in the genus *Choristoneura.* The morphological differences between *C. orae* and *C. pinus* and the difference in sex pheromone between *C. orae* and the other western species confirm that *C. orae* is a valid species. Conceivably *C. orae, C. pinus,* and, possibly, *C. viridis* arose from a common ancestor and diverged through allopatric evolution; but the similarity of the sex pheromones among eastern and western species may be an example of parallel evolution, *C. orae, C. biennis,* and *C. occidentalis* evolving as sibling species in the west, *C. pinus* and *C. fumiferana* as sibling species in the east.

The fact that *C. fumiferana, C. occidentalis,* and *C. biennis* all have the same or very similar pheromones raises questions concerning their relationship and possible isolating mechanisms. The range of the three species is not clearly defined at present, and there may be areas of contact. However, the potential for hybridization is reduced by ecological and temporal isolation and possibly also by the presence of secondary chemicals in the sex pheromones. The true relationship of these species will have to await detailed studies in the areas of potential contact.

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Density Gradient Separation of Marrow Cells Restricted for Antibody Class

Abstract. *Primitive cells of (C3H × C57BL/10)F_1 mouse bone marrow, participating with thymocytes in immune responses to sheep erythrocytes, are already committed to the immunoglobulin M or immunoglobulin G antibody class. By equilibrium centrifugation in discontinuous gradients of bovine serum albumin, cells responsible for production of IgM immunocytes migrate to the denser regions, whereas those responsible for IgG immunocytes remain in the lower density regions.*

Although it is generally accepted that individual immunocytes synthesize antibody of a single molecular class (*1*), it is still debated at which stage of differentiation precursor cells become class restricted (*2, 3*). In the mouse, splenic precursors of hemolytic plaque-forming cells (PFC) induced by sheep erythrocytes (SRBC) are not pluripotent. Results of cell transfer experiments indicated (i) that splenic antigen-sensitive units generate direct (IgM) and indirect (IgG) PFC independently of each other (*2*); and (ii) that in the course of immunization, splenic memory units for different subclasses of indirect (IgG) PFC develop independently (*3*). If, however, splenic antigen-sensitive units were pluripotent, as proposed by Nossal *et al.*, by Papermaster, and by Sterzl (*4*), "genetic or phenotypic shifts" within a clone must be postulated to account for the sequential production of different classes of antibody during immune responses. Attempts to identify cells simultaneously producing two classes of antibody were not successful, even during the critical times of transitions from IgM to IgG, or from γG_1 to γG_2 responses (*1*). In addition, the frequent occurrence of splenic focal areas containing direct and indirect PFC, reported by Papermaster (*4*) in support of "genetic or phenotypic shifts," became meaningless once it was realized that splenic precursor units for direct PFC are several times more frequent than the units for indirect PFC (*2*), and bound to overlap.

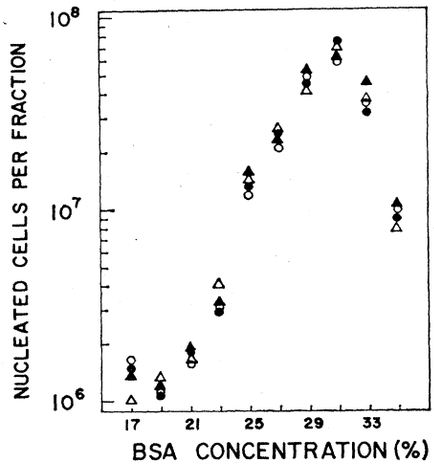

Fig. 1. Density distribution profile of nucleated cells of (C3H × C57BL/10)F_1 mouse bone marrow. 2.5×10^8 cells were layered on the top of the gradient. Symbols indicate individual separations.

In this report, new direct evidence is presented in support of class restriction of precursor units. Bone marrow cells capable of generating either direct or indirect PFC were separated by equilibrium density-gradient centrifugation. Cells were harvested from the long bones of 12-week-old (C3H/He×C57-BL/10)F_1 female mice, suspended in Eagle's medium, and subjected to equilibrium centrifugation in a discontinuous gradient of bovine serum albumin (BSA), according to Dicke *et al.* (*5, 6*). Five to ten cell fractions, designated 1 to 10, were collected from the interphases, beginning at the top, between 17 and 19 percent BSA. The fractions were diluted in 4.7 ml of Ca- and Mg-free phosphate-buffered (*p*H 7.2) saline (PBS), centrifuged at 1000 rev/min for 10 minutes at 10°C, and resuspended in PBS. Nucleated cells were counted with an electronic particle counter (*7*). Density distributions of cells are presented in Fig. 1. The profiles were reproducible and displayed one major peak in the denser region of the gradient. The proportion of cells recovered per fraction was 0.5 to 2 percent in the lower density region (17 to 23 percent BSA), 5 to 10 percent in the intermediate region (25 to 27 percent BSA), and