notype occurs to varying extents in different genetic backgrounds. In *D. mauritiana*, in which this *Mos* factor was first discovered, the maternal effect is undetectable. This may be related to the presence of more than 20 copies of *mariner* in the genome of this species. Strains of *D. simulans* into which w^{pch} and *Mos* have been introduced by repeated backcrossing typically contain fewer than ten copies of *mariner* (*10*). Among four mosaic strains of *D. simulans*, all derived by independent backcrosses from males of the E25H mosaic strain of *D. mauritiana*, the penetrance of the maternal effect was estimated at 13 ± 2, 87 ± 2, 94 ± 1, and 97 ± 1 percent ($\bar{X} \pm SD$) among non-*Mos* offspring. Although the penetrance does not correlate in any obvious way with the number of copies of *mariner* in the strains, it does correlate with the phenotype of the mosaicism. Strains in which the mosaicism is manifested as numerous large pigmented sectors show the highest penetrance of the maternal effect, whereas mosaic strains with a salt-and-pepper pattern of small pigmented spots have the lowest penetrance.

The maternal transmission described here appears distinct from the cytoplasmic transmission described in P-M and I-R hybrid dysgenesis. The major difference is that, in the case of w^{pch} mosaicism, maternal transmission involves factors that enhance the excision of *mariner*; whereas, in hybrid dysgenesis, maternal transmission involves factors which repress excision. In the case of w^{pch}, the eye-color mosaicism provides a convenient phenotypic assay for excision, which may enable further detailed analysis of maternally transmitted substances affecting this process.

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Hawaiian Courtship Songs: Evolutionary Innovation in Communication Signals of *Drosophila*

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In Hawaii, flies of the genus *Drosophila* have undergone spectacular adaptive radiation, resulting in the evolution of more than 500 species of *Drosophila* that are found nowhere else on earth. This taxonomic uniqueness is reflected in behavior and morphology. Hawaiian *Drosophila* sing songs, as do continental *Drosophila*; however, the Hawaiian songs have diverged strongly in form and mechanism of production. The click-song of *D. fasciculisetae* (Maui) has a carrier frequency an order of magnitude higher than those reported in familiar continental species, such as *D. melanogaster* (170 hertz). *Drosophila fasciculisetae*'s song resembles a cicada's more than a fly's song. The song of *D. cyrtoloma* (Maui) has a complex pulse rhythm more typical of crickets than flies. The pulse song of *D. silvestris* (Hawaii) closely resembles that of *D. melanogaster* in both pulse rhythm and carrier frequency, but *D. melanogaster* sings by vibrating its wings, whereas *D. silvestris* sings through abdominal vibrations. These mechanisms are radical departures from the continental wing song mechanism and are further examples of the remarkable behavioral innovation that has occurred in the *Drosophila* of Hawaii during their evolutionary transit through these islands.

HAWAII IS THE MOST ISOLATED island archipelago on earth. New islands are periodically generated by ongoing volcanic activity and provide virgin habitats for new colonization; this, coupled with isolation, has permitted the evolution of more than 500 species of native *Drosophila* (*1*). Their descent can be traced by studies of chromosomes, morphology, and behavior to one or two continental founder-females (*2*). By adaptive radiation the Hawaiian *Drosophila* have diverged widely from continental species in both morphology and behavior. We present song recordings of Hawaiian *Drosophila* and report the striking differences in the acoustic properties of these songs as well as their mechanism of production, compared to the well-characterized songs of continental *Drosophila* species, with which they share common ancestry.

We describe courtship songs of three species, *D. silvestris* (Hawaii island), *D. fasciculisetae* (Maui), and *D. cyrtoloma* (Maui) (*3*). Single males of each species were introduced into recording chambers containing one to three conspecific females, and their songs were tape-recorded and analyzed (*4*). We have recorded songs from 20 of the 106 species of Hawaiian picture-winged *Drosophila*. They can be classified into four distinct acoustic types: (i) click-trains, (ii) complex pulse-trains, (iii) simple pulse-trains ("purrs"), and (iv) simple tone songs

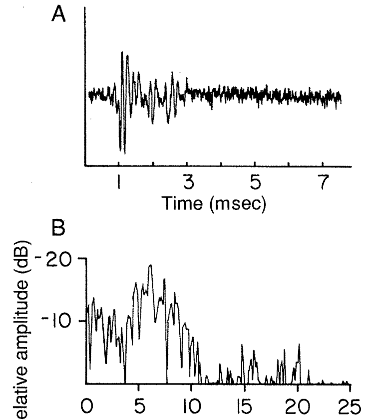

Fig. 1. Analysis of a single click-pulse from the courtship song of *D. fasciculisetae*. (**A**) The time signal (oscillogram) of a single click. (**B**) Relative amplitude spectrum of a click as determined by FFT analysis. The "noisy" nature of the click is apparent from the multiple peaks in the frequency spectrum, although a dominant peak in the spectrum, in the range from 5 to 6 kHz, is characteristic of songs of this species. The frequency response of the recording equipment is limited to 15 kHz [see (*5*)].

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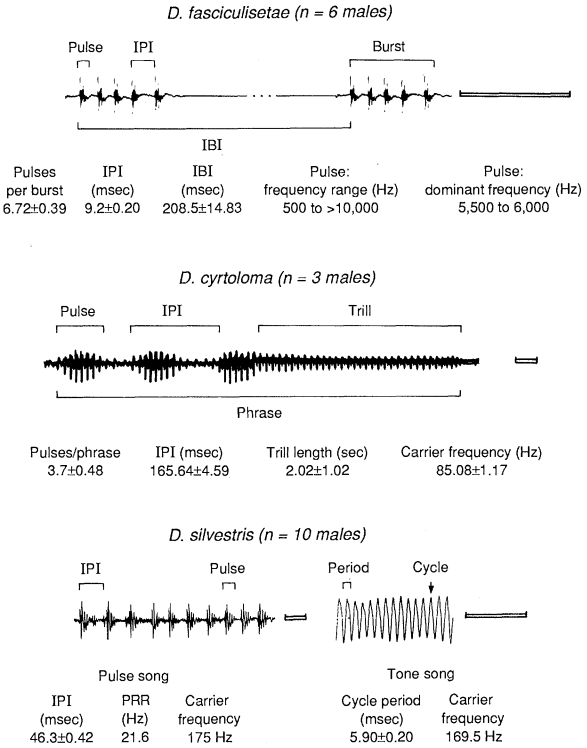

Pulses per burst	IPI (msec)	IBI (msec)	Pulse: frequency range (Hz)	Pulse: dominant frequency (Hz)
6.72±0.39	9.2±0.20	208.5±14.83	500 to >10,000	5,500 to 6,000

Pulses/phrase	IPI (msec)	Trill length (sec)	Carrier frequency (Hz)
3.7±0.48	165.64±4.59	2.02±1.02	85.08±1.17

IPI (msec)	PRR (Hz)	Carrier frequency	Cycle period (msec)	Carrier frequency
46.3±0.42	21.6	175 Hz	5.90±0.20	169.5 Hz

Fig. 2. The courtship songs of three species of picture-winged Hawaiian *Drosophila.* Oscillogram traces of their songs are shown. Below each song trace are listed the summary statistics (mean ± SEM) of relevant acoustic parameters depicted in the oscillogram. These are defined as follows. Pulse: multicyclic sound unit; IPI: interpulse interval, the time interval between successive pulses; IBI: interburst interval, the time interval between successive bursts; and PRR: pulse repetition rate. All songs have a pulse structure except for the tone song of *D. silvestris*, which is a sinusoidal-like progression of sound cycles. For this song type, cycle period refers to the interval between successive cycles, the reciprocal of which is the carrier frequency. In the tone song depicted the amplitude of the tone fluctuates, but this does not occur in all males. A 50-msec time scale appears to the right of each sound trace. In our samples the between-specimen variation within each species was small enough so that the SEM was adequately approximated by $(SD/\sqrt{n})$.

("hums"). Hawaiian and continental *Drosophila* courtship songs are emitted by courting males at very close range to the female (*5*).

The first song type, represented by *D. fasciculisetae*, is composed of repeated, short bursts of click-like pulses (Figs. 1A and 2). The power spectrum of individual clicks is distributed over a wide range of frequencies, ranging from 500 Hz to greater than 10 kHz, with a peak typically occurring around 6 kHz (Fig. 1B). By contrast, in the songs of *D. melanogaster* and other continental species, the peak frequency occurs in the range from 100 to 600 Hz (*6*). High-frequency clicks commonly occur in the songs of cicadas and katydids, in which they are produced by mechanisms that perform frequency multiplication, such as stridulatory apparatus or a muscle-activated tymbal (*7*). However, high-frequency (kilohertz) sounds have not been reported in continental *Drosophila*, or even the order Diptera, hence they may be a Hawaiian innovation. Mechanistically, the songs of *melanogaster* and *fasciculisetae* appear to be variations on a common "theme." Wing movements are associated with sound production in both species. However, *melanogaster*'s sound pulses are generated by movements of the entire wing vane (*6*), whereas *fasciculisetae*'s clicks can continue after amputation of most of the wing, sparing only short wing-stubs (*8, 9*). The mechanism that generates the carrier frequency is clearly different in *fasciculisetae*. We have recorded seven other "click-species" of picture-winged *Drosophila* from the pilimana, vesciseta, and grimshawi subgroups (*9*).

The second song type, exemplified by *D. cyrtoloma*, has a complex temporal pattern (Fig. 2), a repeating phrase that consists of two rhythms: (i) a series of two to seven polycyclic pulses that have a characteristic interpulse interval (IPI) and (ii) a long series of stereotyped sound cycles, the trill. The pulses are always more intense than the trill. Although *cyrtoloma*'s song is rhythmically complex, some continental *Drosophila* also sing complex song rhythms (*10*). *Drosophila cyrtoloma* males sing in the same behavioral context as *fasciculisetae*. The male stands behind the female with his head under her wings; he extends his wings and beats them in the two-part rhythm for many seconds until the female accepts him or decamps. Complex wingbeat rhythms occur in at least six other species of the planitibia subgroup, a closely related complex of 15 picture-winged species (*9*).

The third and fourth song types are exemplified by *D. silvestris*. The simple pulse song, or purr, is a train of polycyclic sound pulses (Fig. 2). The purr has a characteristic interpulse interval, as occurs in the pulse songs of continental *Drosophila* (*11*). Although *silvestris*'s purr sounds remarkably similar to *melanogaster*'s pulse song in both temporal pattern (IPI) and carrier frequency (*6*), they are generated by different mechanisms. *Drosophila melanogaster* sings by wing vibration (*12*), whereas *silvestris*'s purr is associated with low-amplitude abdominal vibrations. In ten cases, *silvestris* males were silenced either by waxing ($n = 6$) the abdomen to thorax (which prevents abdominal movements) or by cutting the dorsal longitudinal and lateral oblique muscles of the first abdominal segment ($n = 4$) (which also eliminates abdominal vibration). Complete amputation of the wings did not prevent courting males from purring ($n = 10$). To our knowledge, sound production by an abdominal "motor" is unknown among continental *Drosophila.*

The fourth song type, the tonal hum, appears to be behaviorally analogous to the songs of *fasciculisetae* and *cyrtoloma*, in that wing movements are associated with sound production. The male stands directly behind the female with his head under her wings; he extends and vibrates his wings rapidly, producing a sinusoidal-like tone (hum). The carrier frequency of the tone is 170 Hz (Fig. 2). Purrs and hums have been recorded in four other closely related species of the planitibia subgroup of Hawaiian picture-winged *Drosophila* (*9*).

Although we have not yet determined whether Hawaiian flies can hear any of these signals, the kilohertz carrier frequencies in *fasciculisetae* songs would be inaudible to the aristal "ears" of continental *Drosophila* (*13*). Aristae are only sensitive in the range from 100 to 500 Hz (*13*) and would be sufficient to detect the songs of *melanogaster*, *silvestris*, and *cyrtoloma* but not *fasciculisetae*'s click, which might have required the evolution of a novel ear to detect them.

Although we have examined only about a quarter of the picture-winged species, some correlations can be made between a fly's song type and its taxonomic position. Hundred-hertz carrier frequency pulse-trains and hums are associated with the primitive planitibia subgroup of the picture-wing *Drosophila*, whereas kilohertz carrier frequency click-trains have been found only in the more advanced pilimana, vesciseta, and grimshawi subgroups. At least seven picture-winged species seem not to produce sounds during courtship.

Our assertion that novel sounds have evolved in some of the Hawaiian *Drosophila* (for example, *fasciculisetae*'s click) is based on comparisons with data for continental species. Our acoustic data include detailed spectral analysis, but few descriptions of continental *Drosophila* include spectral analysis. Spectral analysis might reveal heretofore undetected features of continental courtship songs and provide clues to the evolutionary relationship between continental *Drosophila* and Hawaiian species.

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Steroid Binding at σ Receptors Suggests a Link Between Endocrine, Nervous, and Immune Systems

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Specific σ binding sites have been identified in the mammalian brain and lymphoid tissue. In this study, certain gonadal and adrenal steroids, particularly progesterone, were found to inhibit σ receptor binding in homogenates of brain and spleen. The findings suggest that steroids are naturally occurring ligands for σ receptors and raise the possibility that these sites mediate some aspects of steroid-induced mental disturbances and alterations in immune functions.

IN 1976, MARTIN AND CO-WORKERS postulated the existence of σ receptors to account for the psychotomimetic effects of certain benzomorphan opioids (*1*). Specific haloperidol-sensitive σ receptor binding sites were first identified in preparations of the guinea pig brain by using [^{3}H]*N*-allylnormetazocine (SKF-10,047) as a radioactive ligand (*2*). In more recent studies, tritiated ethylketocyclazocine, haloperidol, 3-(3-hydroxyphenyl)-*N*-(1-propyl) piperidine, and 1,3-di(2-tolyl)guanidine have been used to label σ receptors (*3*). Data from autoradiographic and biochemical studies suggest that the σ receptors are not D$_2$ dopamine receptors, for which most antipsychotic drugs, including haloperidol, have a high affinity (*3, 4*). There is also a distinction between the σ and phencyclidine (PCP) receptors (*5, 6*), although many drugs bind to both sites. The fact that prototypic σ receptor ligands and PCP alter mood and produce hallucinations suggested that the endogenous σ system may play a role in the etiology of human psychosis (*7, 8*). Nonetheless, the physiological role of the σ receptor remains unknown.

The search for endogenous σ receptor ligands has yielded guinea pig brain extracts with selective activity at σ receptors (*8, 9*). Partial purification and chemical characterization indicated that the active material is nonpeptidic and has a mass of 300 to 700 daltons (*8, 9*). The material potentiated electrically stimulated contractions of the guinea pig vas deferens in vitro, a bioassay for σ receptor ligands (*8–10*).

The recent discovery of σ receptors on human peripheral blood lymphocytes and in the rat spleen (*11*) and the hypothesis that σ receptors mediate psychotomimetic responses suggested that an endogenous σ ligand might affect immune function, cause psychosis, and alter mood. Gonadal and adrenal steroids have molecular weights that are in the approximate range of σ-active brain extracts (*8–10*). They can also influence humoral and cell-mediated immunity (*12*) and appear to have a central nervous system action, with complex effects on behavior and mood (*13, 14*). We therefore examined the interaction of σ receptors in the brain and spleen with 20 of the representative gonadal and adrenal steroids. Two putative σ receptor antagonists, haloperidol (*10*) and BW 234U (*15*), were also tested. We report here that progesterone, testosterone, desoxycorticosterone, and several other steroids are potent ligands at σ receptors in the brain and spleen. The potencies of these steroids in binding to σ receptors are also compared with published findings on their efficacies in preventing granuloma formation in rats (*16*).

Brain and spleen tissue was obtained from male Hartley guinea pigs (300 to 400 g). Animals were killed by carbon dioxide asphyxiation, and homogenates were prepared from the excised tissues (*17*). Two-milliliter aliquots of the brain homogenates were incubated with varying concentrations of the inhibitors and 2 n*M* *d*-[^{3}H]SKF-10,047 [23 Ci/mmol; New England Nuclear (NEN)]. Specific binding was defined as that

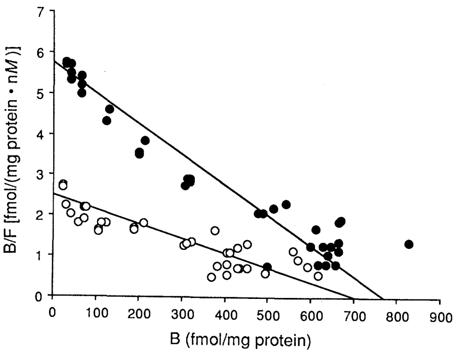

Fig. 1. Scatchard plot of *d*-[^{3}H]SKF-10,047 binding to cerebral σ receptors in the absence (closed symbols) and presence (open symbols) of 800 n*M* progesterone. Increasing concentrations of *d*-[^{3}H]SKF-10,047 (5 to 800 n*M*) were incubated, in the absence and presence of progesterone, with guinea pig brain homogenates in a total volume of 2 ml. Each determination was performed in duplicate and was repeated four times. Data from all four determinations were combined for statistical analysis by the LIGAND computer program (*19*).

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