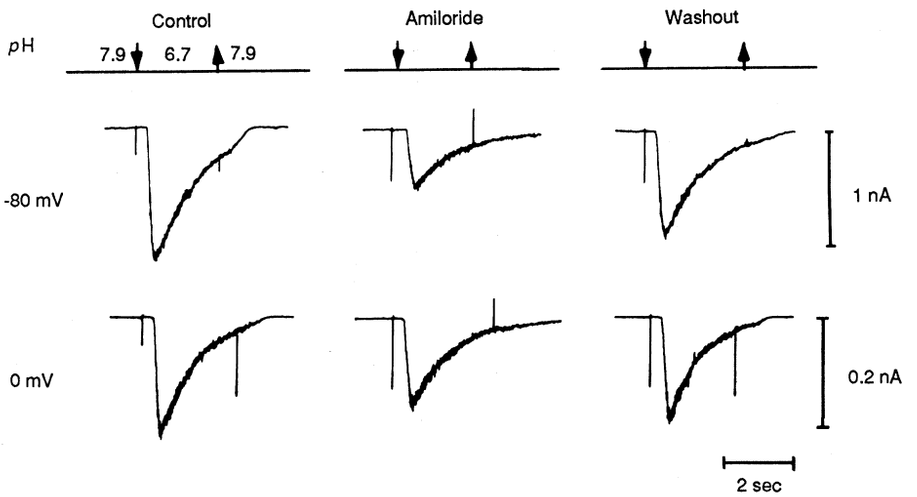

Fig. 4. Voltage-dependent block of $I_{Na(H)}$ by amiloride. In a voltage-clamped neuroblastoma cell, rapid increase of the extracellular proton concentration from *p*H 7.9 to 6.7 activates a transient inward Na^+ current which can be deactivated when the proton concentrations are step reduced to *p*H 7.9 (upward arrow). Amiloride blocks this current in a voltage-dependent manner (middle panels). At a holding potential of −80 mV, 10 μ*M* of amiloride suppressed approximately half of the current, while there is only minimal suppression at 0 mV. The external solution contained 140 m*M* Na^+, 1 m*M* Ca^{2+}, 10 m*M* Pipes and 2 μ*M* TTX. The internal solution contained 80 m*M* Cs^+, 20 m*M* TEA, 20 m*M* Na^+, 20 m*M* Hepes, and 10 m*M* EGTA and was buffered to *p*H 7.3.

impulse conduction through the atrioventricular node and suppressed the pacing rate in the sinoatrial node (*22, 23*). Because the T-type Ca^{2+} channel appears to mediate pacing in heart cells (*24*), we tested the effect of amiloride on I_{Ca} and found that it selectively blocked the T-type current in guinea pig atrial myocytes (*25*).

The suppressive effect of amiloride on the Ca^{2+} current is shared by a number of its chemical derivatives. In particular, the addition of substituted benzyl moieties to the guanidine side group greatly enhanced the blocking properties of amiloride derivatives ($K_D < 10^{-6}M$). Thus amiloride and its derivatives can serve as specific inhibitors of the T-type Ca^{2+} channel, making it possible to dissect the physiological role of this channel in different neuronal and muscle tissues.

REFERENCES AND NOTES

1. M. C. Nowycky, A. P. Fox, R. W. Tsien, *Nature (London)* **316**, 440 (1985).
2. R. Llinas and Y. Yarum, *J. Physiol. (London)* **315**, 569 (1981).
3. E. Carbone and H. D. Lux, *Nature (London)* **310**, 501 (1984).
4. R. Mitra and M. Morad, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 5340 (1986).
5. W. Boll and H. D. Lux, *Neurosci. Lett.* **56**, 335 (1985).
6. R. J. Miller and S. B. Freedman, *Life Sci.* **34**, 1205 (1984).
7. A. P. Fox, M. D. Nowycky, R. W. Tsien, *J. Physiol. (London)* **394**, 149 (1987).
8. L. D. Hirning *et al.*, *Science* **239**, 57 (1988).
9. Y. Yaari, B. Hamon, H. D. Lux, *ibid.* **235**, 680 (1987).
10. T. Narahashi, A. Tsunou, M. Yoshi, *J. Physiol. (London)* **383**, 231 (1987).
11. O. P. Hamill *et al.*, *Pfluegers Arch.* **391**, 85 (1981).
12. P. S. Aronson, *Annu. Rev. Physiol.* **47**, 545 (1985).
13. E. Carbone and M. Morad, unpublished data.
14. J. B. Lansman, P. Hess, R. W. Tsien, *J. Gen. Physiol.* **88**, 321 (1986).
15. W. Almers and E. W. McCleskey, *J. Physiol. (London)* **353**, 585 (1984).
16. A. Konnerth, H. D. Lux, M. Morad, *ibid.* **386**, 603 (1987).
17. N. E. Davies, H. D. Lux, M. Morad., *ibid.* **398**, 195 (1988).
18. L. Palmer, *J. Membr. Biol.* **80**, 153 (1984).
19. S. Saribon-Sohraby and D. Benos, *Am. J. Physiol.* **250**, C175 (1986).
20. G. D. Schellenberg, L. Anderson, P. Swanson, *Molecular Pharmacol.* **24**, 251 (1983).
21. T. Tanabe *et al.*, *Nature (London)* **328**, 313 (1987).
22. S. Yamashita, S. Motomura, N. Taira, *J. Cardiovasc. Pharmacol.* **3**, 704 (1981).
23. A. Pousti and M. A. Khoyi, *Arch. Int. Pharmacodyn.* **242**, 222 (1979).
24. N. Hagiwara, H. Irisawa, M. Kameyama, *J. Physiol. (London)* **395**, 233 (1988).
25. M. Naebauer, I. Dukes, M. Morad, unpublished data.
26. Return of the external solution to the normal external solution containing 5 m*M* $CaCl_2$, 140 m*M* NaCl, 10 m*M* Hepes, and 5 μ*M* TTX brought back the usual amiloride-sensitive Ca^{2+} current (not shown in figure).

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Maternally Inherited Transposon Excision in *Drosophila simulans*

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A mutation in the *white* gene of *Drosophila mauritiana* resulting from insertion of the transposable element *mariner* exhibits genetic instability in germline and somatic cells. The instability is greatly enhanced in the presence of the trans-acting autosomal factor *Mos*, giving eye-color mosaics with pigmented sectors of tissue on an otherwise peach-colored background. The *Mos* factor, when introduced into the genome of the sibling species *Drosophila simulans*, exhibited a dramatic maternal effect on expression of the mosaic phenotype. When *D. simulans* mosaic females (heterozygous for *Mos*) were crossed with non-mosaic males, two distinct classes of mosaic offspring occurred, one resulting from a maternal effect in which the non-*Mos* offspring were nevertheless mosaic. The maternal effect was mediated by a product acting after fertilization, and was expressed to varying extents in different backcross strains.

CYTOPLASMIC TRANSMISSION OF SUBstances regulating the mobilization of transposable elements is an important feature of hybrid dysgenesis in *Drosophila melanogaster* (*1, 2*). In hybrid dysgenesis, unidentified factors transmitted through the maternal cytoplasm can suppress mobilization of the transposable P element (*1*). We have found a maternal effect, involving the dominant autosomal factor *Mos*, that is clearly distinct from hybrid dysgenesis in that maternally transmitted cytoplasmic factors enhance excision of the *mariner* element. The two effects are perhaps related in that the excision depends on factors transmitted through the egg.

The *white-peach* (w^{pch}) allele contains the 1.3-kb *mariner* transposable element inserted immediately upstream of the first exon of the X-linked *white* gene (*3*). The genome of *D. mauritiana* contains about 20 copies of *mariner*, one of which is the *Mos* factor (*4*). In combination with *Mos*, excision of the *mariner* element from the w^{pch} allele occurs at high frequency in somatic and germinal cells in *D. mauritiana*. Somatic excision results in mosaic eye-color with pigmented patches on an otherwise peach-colored back-

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ground (*5*). *Mos* was originally identified because it was found to be deleted in mutant strains that were no longer mosaic (*4*). This copy of *mariner* may cause mosaicism either because of its position in the genome or because of nucleotide substitutions it contains.

To enable genetic mapping of the *Mos* factor, both *Mos* and the unstable w^{pch} allele were transferred from *D. mauritiana* into *D. simulans* by a series of backcrosses. The interspecific cross yields fertile female hybrids, which were backcrossed to *D. simulans* males in each generation. While performing crosses to map the *Mos* factor, we observed that females of genotype *Mos*/+ showed apparently anomalous transmission of the mosaic phenotype. When *Mos*/+ females, homozygous for w^{pch}, were crossed with w^{pch} males from a non-mosaic strain, all offspring of both sexes were found to be mosaic. However, only about 50% of the mosaic progeny transmitted the mosaicism to their offspring, a finding consistent with there being *Mos*/+ and +/+ progeny in the ratio 1:1. Moreover, the *Mos*/+ mosaics, which transmit the mosaicism, were phenotypically distinguishable from the +/+ mosaics, which produce exclusively non-mosaic w^{pch} progeny (Fig. 1). The *Mos*/+ mosaics exhibit a pattern of mosaicism characterized by frequent reversion events at different stages of eye development (Fig. 1a). The +/+ (maternal) mosaics show very few, presumably early, reversion events, giving rise to large sectors of pigmented tissue (Fig. 1b). Although male mosaics with the phenotype in Fig. 1a transmit the mosaicism to half their offspring (*6*), males with the phenotype in Fig. 1b do not (*7*). Since the offspring of *Mos*/+ fathers include mosaic and non-mosaic progeny in the expected 1:1 ratio, the mosaicism among the non-*Mos* progeny of heterozygous females must result from maternal transmission.

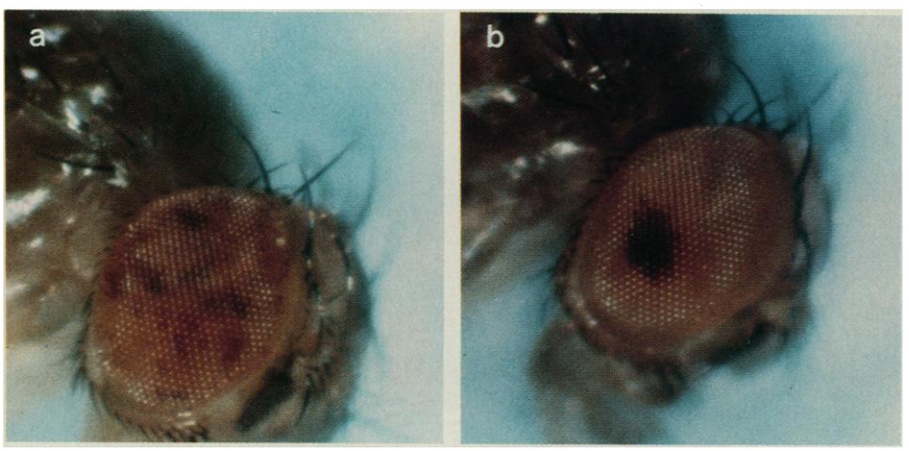

Fig. 1. The two types of mosaic eye phenotype seen in progenies of *D. simulans* females heterozygous for the mosaicism factor. (**a**) A "heritable" mosaic, carrying the genomic factor that promotes somatic excision of *mariner* from *white*, genotype *Mos*/+. (**b**) A fly lacking the heritable genomic factor (genotype +/+), but nevertheless an eye-color mosaic. This latter type of mosaicism, showing few reversion events early in eye development, is typical of the maternal-effect phenotype.

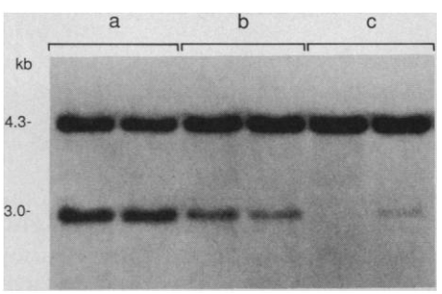

Fig. 2. DNA hybridization analyses in heritable and maternal-effect mosaics. Flies heterozygous for the mosaicism factor (*Mos*/+) were crossed individually to flies from a non-mosaic w^{pch} strain. Both reciprocal crosses were performed. Crosses with *Mos*/+ males yielded approximately equal numbers of mosaics and non-mosaics (19 heterozygous males tested, average of 74 progeny per male). Crosses involving *Mos*/+ mothers generated mosaic progeny of the two types shown in Fig. 1 in approximately equal numbers (17 heterozygous females tested, average of 62 progeny per female). From the progenies of the latter cross, single females were mated to w^{pch} males and allowed to oviposit for 6 to 7 days, at which time the females were used to prepare genomic DNA. The progenies of these females were subsequently scored for the presence of mosaicism in order to confirm correct classification. Genomic DNAs were digested with Bam HI, separated on a 0.7% agarose gel, and transferred to a nylon membrane. The filter was hybridized with a ^{32}P-labeled, 3.0-kb Bam HI fragment of the wild-type *white* locus, which includes the site into which *mariner* is inserted in the w^{pch} mutation. See (*3, 5*) for restriction map. In Southern blots, w^{+} flies show a single band at 3.0 kb, whereas flies homozygous for w^{pch} give a single band at 4.3 kb; the 1.3-kb difference in size corresponds to the size of the *mariner* insert. Mosaic flies typically show both bands, demonstrating somatic excision of *mariner* from *white* (*5*). Lanes are as follows: a, heritable mosaics; b, maternal-effect mosaics; c, w^{pch} flies randomly selected from the non-mosaic strain. Each lane contains DNA from ten female flies (approximately 3 μg). Procedures for isolation of genomic DNA, Southern transfer, labeling of DNA to high specific activity by random oligonucleotide-primer extension, and filter hybridization have been described (*11–14*).

The mosaic phenotype in the presence of *Mos* results from somatic excision of *mariner* from the w^{pch} allele (*5*). To determine whether the maternally inherited mosaicism also results from somatic excision, mosaics of both heritable and maternal types were examined by DNA hybridization analysis (Fig. 2). Although both types of mosaic show somatic excision of *mariner* from the *white* gene, the heritable mosaics (Fig. 2a) show a greater extent of excision than observed in maternal-effect mosaics (Fig. 2b). All flies classified as heritable or maternal mosaics were progeny-tested to confirm that they had been correctly scored. Occasional examples from the *D. simulans* w^{pch} strain exhibited a low background level of spontaneous excision of *mariner* from *white* (Fig. 2c). However, the level of excision in the w^{pch} strain of *D. simulans* was much lower than that seen in the flies with the maternal pattern of mosaicism. This difference corresponds to a phenotypic difference in the extent of mosaicism. While mosaic flies were occasionally seen in the *D. simulans* w^{pch} strain, the pigmented sectors were almost invariably small, and usually single ommatidia.

The maternal-effect mosaics from w^{pch}/w^{pch}; *Mos*/+ mothers receive not only maternal cytoplasm but also a maternally derived w^{pch} allele. Therefore, it is possible that the maternal w^{pch} allele undergoes a genetic imprinting that results in the excision of *mariner*, even in the absence of *Mos* or its products. In maize, an imprinting phenomenon has been observed in studies of the *suppressor-mutator* transposable element system (*8*). The possibility of genetic imprinting was tested by crossing mosaic males, homozygous for *Mos*, to an attached-X strain that carries a stable *white* allele and lacks detectable eye pigment. The female progeny of this cross, heterozygous for *Mos*, were then mated to males of a non-mosaic w^{pch} strain. Male progeny from the latter cross must receive a w^{pch} allele from their father. Fifteen heterozygous females were tested, and all gave non-*Mos* male progeny, showing the maternally transmitted type of mosaicism as well as *Mos* progeny in approximately equal numbers (*9*). We conclude that the maternal-effect mosaicism results from maternal transmission of a factor promoting excision of *mariner* rather than imprinting of the maternal w^{pch} allele.

Maternal transmission of the mosaic phe-

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 ($\bar{X}$ ± 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.

REFERENCES AND NOTES

1. W. R. Engels, *Annu. Rev. Genet.* 17, 315 (1983).
2. J. C. Bregliano and M. G. Kidwell, in *Mobile Genetic Elements*, J. A. Shapiro, Ed. (Academic Press, New York, 1983), pp. 363–410.
3. J. W. Jacobson, M. M. Medhora, D. L. Hartl, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8684 (1986).
4. M. M. Medhora and D. L. Hartl, unpublished observation.
5. G. J. Bryan, J. W. Jacobson, D. L. Hartl, *Science* **235**, 1636 (1987).
6. A total of 55 males were tested by crossing to w^{pch} females; total number of mosaics = 1738, total number of non-mosaics = 1634, $P \cong 0.08$ as determined by a χ^2 test for goodness of fit to 1:1.
7. A total of 60 males were tested; total number of w^{pch} progeny = 4397.
8. N. Fedoroff, in *Mobile Genetic Elements*, J. A. Shapiro, Ed. (Academic Press, New York, 1983), pp. 1–63.
9. Number of *Mos*/+ = 179, number of +/+ = 195.
10. G. J. Bryan and D. L. Hartl, unpublished observation.
11. J. T. Lis, J. A. Simon, C. A. Sutton, *Cell* **35**, 403 (1983).
12. T. Maniatis, E. F. Fritsch, J. Sambrook, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1982).
13. E. M. Southern, *J. Mol. Biol.* **98**, 503 (1975).
14. A. P. Feinberg and B. Vogelstein, *Anal. Biochem.* **132**, 6 (1983).

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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

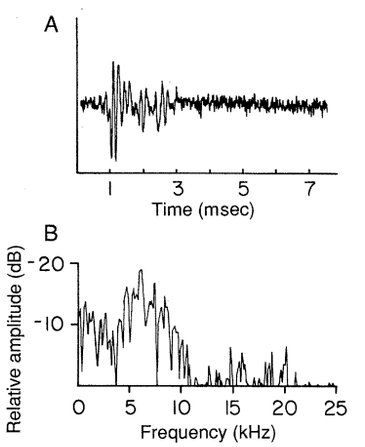

Fig. 1. Analysis of a single click-pulse from the courtship song of *D. fasciculisetae*. (**A**) The time signal (oscillogram) of a single click. (**B**) Relative amplitude spectrum of a click as determined by FFT analysis. The "noisy" nature of the click is apparent from the multiple peaks in the frequency 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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