5. C. Heymans and E. Neil, *Reflexogenic Areas of the Cardiovascular System* (Churchill, London, 1958).
6. J. H. Comroe, Jr., in *Handbook of Physiology*, section 3, *Respiration*, W. O. Fenn and H. Rahn, Eds. (Amer. Physiol. Soc., Washington, D.C., 1964), vol. 1, p. 557.
7. Sponsored by the European office of USAF Office of Scientific Research, OAR (grant AF EOAR 65-6), and by Consiglio Nazionale delle Ricerche (electrophysiology group).

3 May 1966

"Sex-Ratio" Condition: Unusual Mechanisms in Bark Beetles

Abstract. *Although males and females occur in similar frequency in the broods of most scolytid beetles, certain individuals of several species produce offspring highly aberrant in the ratio of sexes—all or mostly female. In some instances this condition reflects gynogenesis (parthenogenesis stimulated by mating); in others, a maternally transmitted factor.*

Determination of sex in scolytid beetles is of the XX-XY type with homogametic females. The sex ratio of broods is usually about 1 : 1, but extreme departures may result from several different mechanisms. We have found individuals in two taxonomic groups that produce only or almost only female progeny. Two entirely different mechanisms are concerned: In *Orthotomicus* (formerly *Ips*) *latidens* a cytoplasmic factor is responsible, whereas in *Ips tridens, Ips englemanni, Ips simirosteris, Ips amiskwiensis*, and *Ips yohoensis* a type of parthenogenesis (gynogenesis) exists.

The phenomenon of "sex ratio" (SR) was first carefully analyzed by Gershenson (*1*) in *Drosophila obscura*, in which a particular X chromosome carried by a male results in production of all-female progeny. An apparently quite similar abnormality was earlier found in *D. affinis* (*2*), but the stock was lost before a detailed study could be made. Sturtevant and Dobzhansky (*3*) showed that the Y chromosomes degenerate regularly in SR strains of *D. pseudoobscura*. Novitiski *et al.* (*4*) found that, contrary to earlier reports, the X chromosomes behave normally. That the Y chromosomes show degeneration at second anaphase suggests that the SR condition can be interpreted in terms of regularly nonfunctional products of meiosis. Another type of mechanism, by which unisexual progeny have been produced in *Drosophila*, and which is caused by cytoplasmic infections, also has been termed SR; these instances have been reviewed by Poulson (*5*) and covered by others (*6*). Poulson and Sakaguchi (*7*) found the infective agent to be a new species of *Treponema*-like spirochete, while a virus seems to be the agent in *D. bifasciata* (*8*). Lanier (*9*) reported a SR condition in *Orthotomicus latidens*.

We induced isolated pairs of beetles to produce brood by introducing them into small, freshly cut logs of their natural host; they were held separately in cardboard food cartons until the progeny were mature. All species deposit one egg in an individual niche on either side of a linear egg gallery in the phloem-cambial area of the tree. The larva makes a discrete mine beginning at the egg niche and terminating in a pupal chamber; thus, when the bark is stripped to recover the F_1 adults, the numbers of egg niches and larval mines are easily determined.

The SR line of *O. latidens* was collected in *Pinus jeffreyi* (*10*); its pedigree appears in Fig. 1. Three of the 11 females of the brood, paired with males of the same population, produced a total of 70 female and no male offspring. Some of the F_2 were paired with males of the same population and some with males from Lake County, about 480 km distant, without effect on the sex ratio of the progeny. Males sometimes appear in early broods of SR females, but subsequent broods from the same females usually contain only females (Fig. 1, F_3 generation). In the F_1 and F_2 generations (Fig. 1) approximately 50 percent of the egg niches yielded larvae; normally 92 percent of eggs produce larvae in the bisexual population. Some initial broods of the F_3 included a few males and had an overall larva : egg-niche ratio of 0.61, while second broods (females only) had a ratio of 0.49 (Fig. 1). The overall ratios in the F_4 generation were 0.47 for all-female broods and 0.82 for SR broods containing males. The male : female ratio for the latter broods was 0.65 : 1, which is almost exactly what we would have predicted from the larva:egg-niche ratio of 0.82 if the reduction in hatchability were attributable to mortality of male embryos (*11*). Unmated females fail to oviposit; those mated to the closely related species *O. sabinianae* oviposit but no eggs hatch (*9*).

The necessity for conspecific matings for progeny, and the very close correlation between egg hatchability and number of males in SR broods, indicate that SR condition in *O. latidens* reflects mortality of the male embryos. The uniform inheritance of the condition through several generations of random and selected matings and the matrilineal nature of the inheritance suggest that male mortality cannot be caused by sex-linked or sex-limited genes per se; rather it must be a factor transmitted in the cytoplasm of the egg. All our results with this species parallel the instances of infected *Drosophila* previously mentioned. Unfortunately attempts to identify an agent from the hemolymph of *O. latidens* with phase-contrast and darkfield microscopy have failed; failure suggests that the causative agent may be a virus rather than a spirochete unless either its distribution in the host is limited or the titers are very low.

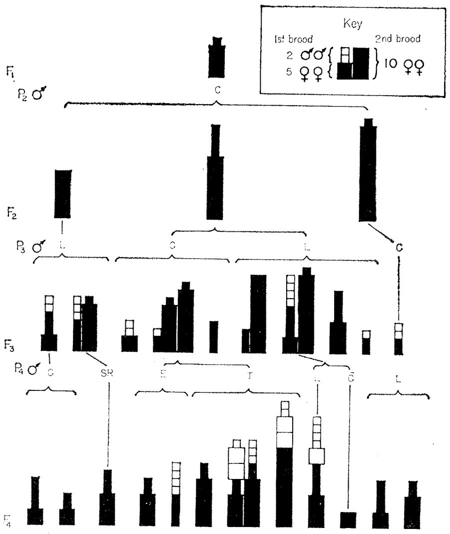

Fig. 1. SR line of *Orthotomicus latidens*, F_1 through F_4. A small square represents one individual; a large square, five. Black, female; white, male. Contiguous histograms sequentially arranged from left to right represent successive broods by the same female (see key). Parent females were from broods indicated by brackets. The parent males came from these Californian counties: Calaveras (*C*) (source of the SR strain), Lake ($\mathcal{L}$), El Dorado (*E*), and Tahoe (*T*); *SR*, from SR brood.

Hopping (*12*) discovered SR strains of four Canadian bark beetles, *Ips tridens, Ips englemanni, Ips simirosteris*, and *Ips amiskwiensis*; in four generations there was no change in all-female broods and apparently no reduction in number of progeny. Smith (*13*), examining Hopping's data, suggested that the females might be gynogenic. We can now confirm that individuals of each of these species and of *Ips yohoensis* are gynogenic; in fact all five "species" probably should be considered a single species with polymorphic females; all males are indistinguishable cytologically or morphologically, but the females differ morphologically.

Matings of males whose sisters are of one morphological form to normal females of another form result in bisexual progeny containing females that are morphologically highly variable. Without exception, however, SR females produce broods of daughters almost exactly like themselves, whether mated to males whose sisters were of the same or a different morphological form, or even to males of two distinctly different species such as *Ips borealis* and *Ips perturbatus*. Regardless of the type of mating, hatchability in SR broods approaches 100 percent, whereas normal bisexual *Ips tridens* mated to *Ips borealis* or *Ips perturbatus* produce eggs of which none hatch. Females, both SR and normal, fail to oviposit unless mated.

This gynogenic condition appeared spontaneously in two laboratory matings of females from bisexual broods. There was no history of gynogenesis in either of the parent lines and outmatings of brothers produced bisexual progeny. Genes for gynogenesis may be floating in certain populations and may be occasionally expressed; once induced, the condition is probably obligatory for the duration of that particular line.

One may wonder how widely spread is the SR condition in the Scolytidae. In addition to the species mentioned, we have discovered gynogenic strains of *Ips borealis* and *Ips perturbatus* and a SR condition in a species of *Pityophthorous*, similar to that in *O. latidens*. Several species of *Xyleborus* are reported to have 15 to 20 females per male in the usual colony (*14*). Nevertheless, not a single SR strain was encountered while we reared several hundred thousand *Ips confusus*.

G. N. Lanier
J. H. Oliver, Jr.
Division of Entomology and Acarology, University of California, Berkeley

References and Notes

1. S. Gershenson, *Genetics* **13**, 488 (1928).
2. T. H. Morgan, C. B. Bridges, A. H. Sturtevant, *Bibliographia Genet.* **2**, 1 (1925).
3. A. H. Sturtevant and Th. Dobzhansky, *Genetics* **21**, 473 (1936).
4. E. Novitski, W. J. Peacock, J. Engel, *Science* **148**, 516 (1965).
5. D. F. Poulson, in *Methodology in Basic Genetics*, W. Burdette, Ed. (Holden-Day, San Francisco, 1963), pp. 404–24.
6. B. Sakaguchi, K. Oishi, S. Kobayashi, *Science* **147**, 160 (1965); H. Ikeda, *ibid.*, p. 1147.
7. D. F. Poulson and B. Sakaguchi, *ibid.* **133**, 1489 (1961).
8. E. A. Leventhal, *Amer. Zoologist* **5**, 649 (1965).
9. G. N. Lanier, *Can. Entomologist* **98**, 175 (1966).
10. From Cottage Springs, Calaveras County, Calif.
11. [0.50 (♂ eggs) − 0.18 (unhatched eggs)] : 0.50 (♀ eggs) → 0.64 ♂ : 1 ♀.
12. G. R. Hopping, *Can. Entomologist* **93**, 1050 (1961); **94**, 506 (1962); **95**, 117 (1963).
13. S. G. Smith, *ibid.* **94**, 941 (1962).
14. W. J. Chamberlin, *The Bark and Timber Beetles of North America* (Oregon State College Cooperative Assoc., Corvallis, 1939), pp. 1–513.
15. Aided by NIH research grant AI-06169-01A1 and by grants from NSF (GB-2792), California State Division of Forestry, and the T. B. Walker and Surdna Foundations. The Canadian Department of Forestry provided certain materials and facilities.

14 April 1966

Evoked Potential Correlates of Generalization

Abstract. *Late components of evoked potentials recorded during the occurrence and nonoccurrence of generalization are different. During generalization the evoked response waveshapes resembled those elicited by the conditioned stimulus during correct performance. The differences are statistically significant.*

When an animal learns to perform a conditioned response upon presentation of a particular conditioned stimulus, we assume that a neural representation of this experience is stored somewhere in the brain. If a novel, but similar, stimulus is subsequently presented to the trained animal, performance of the previously learned response sometimes occurs and is referred to as generalization. To explain this phenomenon, it seems reasonable to suggest that the novel stimulus recalls a stored representation of prior experience.

Electrophysiological recordings, taken from electrodes chronically implanted in cat brains, have been reported (*1*) to contain rhythmic activity at two frequencies, when generalization occurs to a novel stimulus at one frequency (F_2) after training with a rhythmic conditioned stimulus at another frequency (F_1). One of the observed frequencies corresponds to the novel stimulus which is actually present (F_2), while the other is approximately that of the conditioned stimulus actually used during training (F_1). Since no physical event at frequency F_1 is present in the environment of the animal, electrical rhythms at F_1 have been assumed to reflect the recall of a neural representation of prior experience from storage in the brain. This interpretation is supported by the observation that rhythms at F_1 are no longer present during F_2 stimulation, after the animal has been taught to differentiate stimuli at the two frequencies.

This report presents some results obtained in more recent studies in which computer analysis was applied to electrophysiological data recorded during generalization. Studies were carried out on four cats, each bearing 34 electrodes chronically implanted in numerous cortical and subcortical brain regions. These animals were trained in performance of a lever-press to avoid shock within 15 seconds after onset of a flickering light (10 cy/sec). After appreciable overtraining, the animals were subjected to test sessions in which occasional trials with a 7.7-cy/sec flicker were interspersed among presentations of the conditioned stimulus of 10 cy/sec. If generalization occurred, the test stimulus was terminated when the lever was pressed. If no behavioral response occurred after 30 seconds, the test stimulus was terminated without electrical shock. Electrophysiological responses obtained in all sessions were

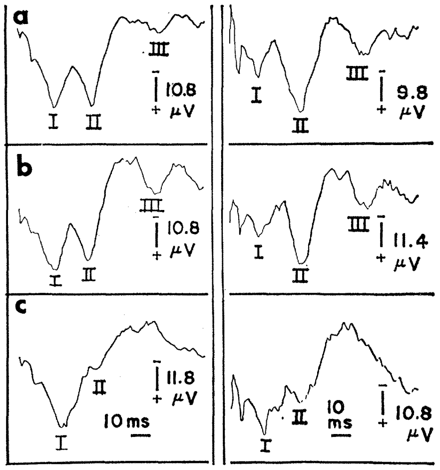

Fig. 1. Average evoked potentials recorded from lateral geniculate body (left) and nucleus reticularis (right) during behavioral trials that resulted in conditioned response performance (*a*) to 10-cy/sec flicker, and generalization (*b*) and no response (*c*) to 7.7-cy/sec flickers. Generalization and no-response averages are based on 42 evoked potentials, and conditioned-response averages on 100 evoked potentials. Analysis epoch is 90 milliseconds.