normal set of varicosities in *Aplysia* serves as a mere scaffolding for behavior. Even such elementary learning experiences as habituation and sensitization can build upon this scaffolding by altering the number and size of active zones to modulate the functional expression of neural connections. Since our observations were made on animals that received long-term behavioral training, these morphological changes may represent an anatomical substrate for memory consolidation.

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9. Animals were individually housed for a minimum of 5 days in circulating seawater before behavioral training. To assess their responsiveness, we delivered two jets of seawater to the siphon with a Water Pik. Animals were accepted for the experiment if the mean duration of their first two test responses (interstimulus interval, 30 seconds) was 10 seconds or larger. The scores (total of ten stimuli) were ranked and the animals were randomly distributed into a control (untrained) group and groups for long-term habituation and long-term sensitization. There were no significant differences among the groups before training. Long-term habituation was produced by giving animals ten sessions of habituation training per day for 10 days. Long-term sensitization was produced by giving animals training sessions on either four or ten consecutive days. For the 10-day protocol a session consisted of exposure to two electrical stimuli (20 mA for 2 seconds), each separated by a minimum of 2 hours. For the 4-day protocol a session consisted of exposure to four electrical stimuli (100 mA for 2 seconds), each separated by 1.5 hours. All electrical stimuli were delivered to the neck region through bipolar capillary electrodes.
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Selection, Outbreeding Depression, and the Sex Ratio of Scale Insects

Abstract. *The black pineleaf scale insect has haploid males and diploid females. Ratios of males to females late in development ranged from 0.005 to 0.320 among insect subpopulations that were infesting different host trees. Demes well adapted to an individual ponderosa pine had a higher proportion of males than did demes that were poorly adapted to the host. Ratios of males to females rose in successive annual samples as natural selection increased insect adaptation. Gene flow between demes on different host trees produced predictable changes in the sex ratio.*

Ponderosa pines (*Pinus ponderosa* Lawson) show great intraspecific variability in their phytochemical defenses (*1*). Because each tree is effectively different (*2*) and because the life history of trees is long relative to the generation time of insect herbivores, pest populations may adapt to individual hosts. Artificial transfer experiments have shown that single pines carry a specialized, differentiated deme or subpopulation of black pineleaf scale insects (*Nuculaspis californica* Coleman) (*3*). We now describe patterns of sex ratio variation in this sedentary insect herbivore which suggest that (i) the differentiation is a genetic process; (ii) drift, or selection adapting scales to the defensive phenotype of an individual host tree, reduces genetic variance within the deme; and (iii) gene flow between scale demes on closely adjacent trees produces a decrease in local adaptation.

Table 1. Male to female (M/F) sex ratios of scale insect demes from 18 different host trees. Samples were taken just before male eclosion in three consecutive years. Tree demes are listed from low to high density (*8*).

1979		1980		1981	
N	M/F	*N*	M/F	*N*	M/F
		327	.073	299	.128
71	.014	409	.073	467	.107
181	.028	347	.089	1037	.101
126	.000	403	.032	192	.091
		327	.052	519	.126
188	.005	412	.068	381	.085
203	.005	337	.113	580	.064
108	.009	368	.098	335	.196
122	.025	382	.055	912	.068
141	.052	208	.087	86	.323
		399	.043	382	.117
147	.007	356	.076	468	.093
76	.101	260	.162	344	.147
		358	.101	462	.079
580	.034	246	.166	398	.223
		366	.109	509	.077
		384	.081	489	.153
		389	.090	414	.092

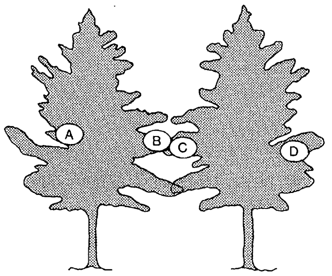
Eclosion of males, flight, and mating of scale insects occur in mid-May on ponderosa pines in northwestern United States. A census of the insects just before males emerge showed wide variation in the male-female ratios among host trees (*4*). Field observations of insect mortality indicated that fewer males than females survive. Sex is determined by a haplodiploid mechanism with obligate fertilization and elimination of paternal chromosomes early in the development of male progeny (*5*). Males carry half as many chromosomes ($n = 4$) as do females ($2n = 8$) and thus are not heterozygous at any loci. We propose that variability in survivorship and sex ratio reflect selection and change in the genetic composition of the subpopulation; genes that are partially deleterious to to survival on a particular tree are eliminated, and genetic variance is reduced. Heterozygous females are protected when deleterious genes are moderately common, but haploid males do not benefit from the presence of homologous alleles.

If our proposal is correct, increases in male frequency in sequential annual samples from a single tree offer a quantitative demonstration of natural selection or genetic drift, as well as a measure of the amount of evolutionary change between insect generations. Data on sex ratios from 18 different tree demes (Table 1) (*6*) show that male frequency increased in all 11 trees sampled during the first year and in 14 of the 18 trees during the second year. The pattern of increasing male frequency is statistically significant in both years (*7*). In addition, the relative abundance of males and the ratio of males to females rise in association with scale density on an individual host (*8*). These data suggest that insect density represents the adaptation of a deme to the defensive character of its host and that yearly changes in sex ratio document the progress of natural selection.

A hypothesis relating sex ratios to selection, drift, and genetic variance also suggests that gene flow between demes should produce changes in male frequency. Gene flow counteracts differentiation. It should increase genetic variance and differences in survivorship between the sexes and decrease the resulting male to female ratio. To examine the impact of gene flow on the sex ratio, we chose 11 pairs of adjacent trees from a heavily scale-infested plot of ponderosa pines and took censuses of insects on near and far sides of the tree crowns. Samples from the near sides were selected where foliage from the two different hosts either touched or was separated by a distance of less than 10 cm. Samples from the far sides were taken from a portion of each tree crown that was separated by at least 5 m from the foliage of any other tree. Although the winged male scales can fly, most mating activity is very localized; males eclose and walk to mates on the same pine needle. Females are sedentary and do not appear to discriminate among mates. We expected samples from near sides to receive more interdemic mating and gene flow than those from far sides and predicted that differences in survivorship would reduce the male to female ratios on near sides in comparison with samples from the far sides on the same tree. Comparison of the data on 19 of 22 pairs (Fig. 1) supported this prediction (*9*).

If survival influences the sex ratio of black pineleaf scale, the insects illustrate an outbreeding depression analogous to Haldane's rule, whereby hybrid inviability is differentially expressed in the heterogametic sex (*10*). The ultimate genetic mechanism that produces these patterns is not known. We have suggested a simple proximate model linking selection or drift and genetic variance to the sex ratio; it is based on increasing adaptive equivalence of the sexes as genetic variation declines within the deme.

We have not yet developed a technique for determining the sex of newly hatched black pineleaf scale larvae. As a result, the relative contributions of primary sex ratio and survivorship to our data are unclear. Although genetic polymorphisms for primary sex ratio are virtually unknown (*11*), there are many examples of facultative or ontogenetic variation. The frequency with which females of the diaspidid scale *Pseudalulacaspis pentagona* (Targ.) lay male eggs increases as the interval between onset of sexual receptivity and mating lengthens (*12*). Local mate competition may also affect sex allocation in demes with a small effective population size (*13*). Each of these mechanisms predicts a pattern different from that displayed by *N. californica* (*14*). Although further study may implicate both primary allocation and secondary survivorship, the extreme bias of our sex ratio data suggests that primary explanations alone are unlikely to be sufficient (*15*). The survivorship model offers a simple hypothesis completely consistent with observed patterns. It has, in addition, a number of interesting implications.



Tree	A		B	C		D	Tree
183	.225	>	.054	.091	<	.112	184
185	.040	>	.030	.035	<	.108	186
187	.171	>	.117	.129	<	.190	188
189	.073	<	.116	.092	<	.113	190
199	.123	>	.054	.097	<	.118	200
26	.135	>	.048	.086	>	.074	27
29	.161	>	.076	.113	<	.146	38
30	.169	>	.076	.077	<	.126	31
32	.106	<	.127	.056	<	.112	33
35	.094	>	.058	.058	<	.069	34
37	.196	>	.153	.052	<	.068	36

Fig. 1. Sex ratios (males to females) from near and far sides of adjacent pairs of trees. Insects were sampled late in their development, just before male eclosion and flight. Each row of data represents four samples from one pair of pines. Columns A and D show sex ratios from far sides and B and C from near sides. Samples from which the ratios were calculated ranged in size from 273 to 852. In 19 of the 22 pairs male frequency is lower on the near side, illustrating an outbreeding depression and reduced male survivorship in response to gene flow. [© Academic Press, New York, in press]

1) Local sex ratios affect the relative abundance of males within a tree and between trees, and hence affect the balance of outcrossing and inbreeding that the deme experiences. Poorly adapted subpopulations in which male mortality is heavy will receive a larger proportion of between-tree matings than demes with a higher frequency of local males. Thus proportionally more outcrossing should promote gene flow during early stages of the infestation on an individual host; later, increased male survival will produce relatively more inbreeding and protect the adaptive characteristics of the deme.

2) Each scale deme must contain a subset of the genes in the whole population. Selection narrowing this subset to meet the defensive challenge of one tree may reduce the range of host chemistries that the insects can tolerate. This coevolutionary model of plant herbivore interaction suggests that adaptation and specificity (of the insects) will be correlated if the processes adapting insects to one host individual reduce the diverse genetic repertoire needed to tolerate a wide spectrum of different defenses in different trees. In artificial transfer experiments in which the survival of scale insects moved from one host pine to another was quantified, high-density demes show less transfer success than low-density demes (*16*).

3) Evolutionary processes that link adaptation and specificity of sedentary insects may affect dietary patterns on a broader level of spatial organization. Many insects are "local specialists"; they feed on a variety of plant species over their entire geographical range but show much more restricted diets within a particular community (*17*). If local populations are selected to tolerate a subset of the plant defenses that are encountered throughout their range, genetic variation and genetic variability may account for the geographic patterns of dietary diversity.

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6. Because the trees were originally chosen to represent a wide range of insect adaptation as part of experiment, some host trees carried high insect densities, and others were more sparsely infested. Colonization of a new host tree by scales usually takes many attempts, and subsequent density increases are very gradual, requiring 20 or more annual generations to achieve a heavy infestation. Scale density during this interval is correlated with increasing fecundity and specificity. By adaptation we mean the process of increasing density, fecundity, and specificity over many generations of selection.
7. Wilcoxon matched-pairs signed-ranks test; for 1979–1980, $P < .001$ and for 1980–1981, $P < .005$.
8. Spearman rank correlations (r) for the 3 years: 1979, $N = 11$, $r = 0.51$, $.10 > P > .05$; 1980, $N = 18$, $r = 0.50$, $.05 > P > .01$; and 1981, $N = 18$, $r = .03$, not significant. Sampling effort was increased after 1979 data showed interesting patterns. Statistical significance of the correlations reflects this change in effort from 1979 to 1980. Rank estimates of density used in all correlations were made in 1977. The declining correlation coefficient illustrates a more rapid change in sex ratio on trees with low density and low male frequency. The 1980 frequency of males is inversely correlated with the 1980–1981 increase of sex ratio on each tree ($r = .48$, $0.05 > P > .01$). Weather affects the survival of scale crawlers and produces variations in density that are independent of selection and host adaptation. All pines in the study area carried low insect densities in 1980 in comparison with 1979 and 1981. The consistently increasing frequencies of males indicate that density alone is not a major determinant of the sex ratio patterns.
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Early Morning Insomnia with Rapidly Eliminated Benzodiazepines

Abstract. *Early morning insomnia, a significant increase in wakefulness during the final hours of drug nights, occurred after 1 or 2 weeks of nightly administration of benzodiazepine hypnotics with short elimination half-lives, when tolerance had begun to develop. Early morning insomnia may be a variant of rebound insomnia and therefore specific to benzodiazepines, or it may occur with any rapidly eliminated sedative-hypnotic agent.*

Benzodiazepine hypnotics with relatively short elimination half-lives have recently received considerable attention, since, compared with long half-life benzodiazepine hypnotics, the short half-life drugs have little accumulation and potentially produce less daytime sleepiness and smaller performance decrements (*1*). As with any drug, however, benzodiazepine hypnotics with relatively rapid elimination rates cannot be uncritically accepted with the assumption that their administration or withdrawal is entirely without undesirable effects. In fact, these drugs produce rebound insomnia, an intense worsening of sleep relative to baseline levels, after they are withdrawn (*2*). This adverse effect occurs even when these drugs are administered in only a single nightly dose for short periods. We now describe a new finding of early morning insomnia that is similarly associated with benzodiazepines having relatively short elimination half-lives. This condition also consists of a worsening of sleep, but it occurs during the final hours of sleep during actual drug administration, whereas rebound insomnia occurs after drug withdrawal. Both phenomena can be interpreted as manifestations of drug withdrawal.

We now present data analyzed from six separate sleep laboratory evaluations of four benzodiazepine hypnotic drugs (*3*). The subjects, whose primary complaint was of chronic insomnia, were continuously monitored by hypnopolygraphic recordings consisting of an electroencephalogram, electromyogram, and electrooculogram (*4*). On every night of these studies each subject received either drug or placebo at "lights out," and then was monitored for an 8-hour period. Throughout the study the subjects were instructed not to nap, not to alter their level of daily physical activity significantly, and not to use any drugs or alcohol.

Two benzodiazepines with short elimination half-lives (*5*) were evaluated: midazolam (20 mg) ($N = 6$) in a 14-night study that included four placebo nights (the first for adaptation and the next three for baseline measurements), 1 week of drug administration, and three placebo-withdrawal nights; and triazolam (0.5 mg) ($N = 7$) in a 22-night study that included four placebo-baseline nights, 2 weeks of drug administration, and four placebo-withdrawal nights (*3*). Two benzodiazepines with long elimination half-lives (*6*), flurazepam (30 mg) and quazepam (30 mg), were also evaluated. For flurazepam, a total of 15 subjects were assessed in three studies, 4 in a 22-night study identical to that described for triazolam and 11 in two separate 47-night studies that included four placebo-baseline nights, 4 weeks of drug administration (for this report, data were analyzed for only the first 2 weeks of drug administration), and 15 placebo withdrawal nights. For quazepam, six subjects were studied in a 47-night study identical to that used for flurazepam (*3*).

For each drug, data were analyzed for two sets of three consecutive drug nights by comparing each of their mean values with the mean value of the set of three baseline nights [Dunn multiple-comparison, two-tailed t-test (*7*)]. For the midazolam study, the first three drug nights (nights 5 to 7) and the last three drug nights (9 to 11) of a 1-week drug administration period were used. With the other three drugs, data were also analyzed from the first three drug nights (5 to 7) and the next available set of three drug nights recorded in the sleep laboratory (nights 16 to 18), which represented the last three drug nights of a 2-week drug administration period. (Because of the design of these studies, sleep laboratory data were not obtained on nights 9 to 11 as they were in the midazolam study.)

All four drugs decreased the time spent awake during the first 6 hours of the night on each of the two sets of three consecutive drug nights. This effect was variable across drugs for the last 2 hours of these drug nights, however. On nights 5 to 7, midazolam decreased mean wake time 37.5 percent below baseline for the first 6 hours (from 47.5 ± 5.5 to 29.7 ± 3.1 minutes, $P < .01$), but during the last 2 hours, only 7.4 percent [from 13.5 ± 4.6 to 12.5 ± 3.2 minutes, not significant (N.S.)]. Triazolam had a similar effect, decreasing wake time 46.6 percent for the first 6 hours (from 84.8 ± 9.2 to 45.3 ± 3.4 minutes, $P < .01$) but only 27.7 percent (from 8.3 ± 1.2 to 6.0 ± 0.9 minutes, N.S.) during the final 2 hours. In contrast, both quazepam and flurazepam were effective in maintaining sleep throughout the