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11. Phytoplankton production in the Aleutians is comparatively low (38 to 243 mg of carbon per square meter per day) concentrated during a relatively short growing season and probably restricted to bays (rather than the exposed coast) with vertical water stability (*9*).
12. Mussels were initially of equal size and were assigned to cages randomly. We could not replicate the "no kelp" treatment of this experiment because we were unable to visit Alaid-Nizki Island until the summer of 1987.
13. All barnacles recruited to the settling plates within a span of several weeks and were of similar size. Barnacle physical dimensions were measured within 48 hours of placement in cages; at that time there was no statistically significant difference in mean basal plate area among islands ($n = 4$) for those individuals that survived to the experiment's conclusion (Kruskal-Wallis, $P > 0.1$). Final dry weight was measured on animals removed from the plates and dried at 50°C for 24 hours.
14. Kruskal-Wallis ANOVA on mean growth of individuals at a site (cage) were mussel intertidal, $\chi^2(2) = 19.3$, $P < 0.0001$; mussel subtidal, $\chi^2(2) = 17.2$, $P < 0.0002$; barnacle dry weight, $\chi^2(2) = 6.4$, $P < 0.05$; barnacle basal plate area, $\chi^2(2) = 6.2$, $P < 0.05$.
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18. See B. Fry and E. B. Sherr [*Contrib. Mar. Sci.* **27**, 15 (1984)] for comprehensive discussion and critique of the application of $\delta^{13}C$ techniques to ecological studies. The ratio of ^{13}C to ^{12}C is fixed at the time (and according to the pathway) of photosynthesis. With minor modification (+0.5 to 1.5 per mil per trophic level), this ratio is maintained through consumer trophic levels. Thus consumer signatures reflect those of key primary producers.
19. Terrestrial input of organic matter to nearshore coastal waters was presumed to be insignificant primarily because most terrestrial vegetation is maritime tundra of grasses and lichens, which degrade in situ; there is no woody vegetation that would produce large amounts of exportable detrital matter.
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21. Five whole (above holdfast) kelp specimens per species were collected at each of three randomly selected sites at each island and subsampled systematically by taking a number of plugs uniformly along the length of the blade. Three to five specimens of each consumer taxon were collected at the same sites as the kelps and were used whole (mysid, amphipod, sea anemone) or subsampled (muscle tissue of others).
22. The single exception to the pattern of greater consumer enrichment at islands with kelps was *Mytilus edulis*, which was the only consumer we collected from the intertidal zone, where kelps are abundant at all four islands. *Mytilus* $\delta^{13}C$ values showed no pattern between kelp and no kelp islands.
23. Differences between kelp and no kelp islands were significant in a random-block ANOVA (with species as blocks, thus allowing analysis of all species simultaneously) considering all subtidal consumers pooled [$F(1,27) = 7.96$, $P < 0.0001$] or only suspension feeders [$F(1,14) = 11.64$, $P < 0.005$].
24. A simple mixing model based upon that of T. McConnaughey and C. P. McRoy [*Mar. Biol.* **53**, 263 (1979)] was possible because of the two-carbon source system. Percentage contribution from kelp is calculated as $[\delta^{13}C \text{ sample} - \delta^{13}C \text{ phytoplankton} - I]/[\delta^{13}C \text{ kelp} - \delta^{13}C \text{ phytoplankton}] \times 100$, where I represents a post-photosynthetic isotope fractionation and was empirically derived for each species by calculating the difference in $\delta^{13}C$ between the most deplete sample of that species ("pure" phytoplankton diet) and the mean phytoplankton value (−24.0). In cases where the most $\delta^{13}C$ deplete value for a species was less than our phytoplankton value, the mean of our measured enrichment values (2.5 per mil per trophic level) was used. This method makes our model conservative in favor of phytoplankton (reducing the percentage of carbon from kelp) in that our calculations indicate that even the most isotopically deplete consumers incorporate some kelp-derived carbon.
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A 48-Million-Year-Old Aphid–Host Plant Association and Complex Life Cycle: Biogeographic Evidence

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Biogeographical and paleobotanical evidence suggests that the aphid subtribe Melaphidina has been associated with its sumac host plant since the early Eocene when these plants were continuously distributed across the Bering land bridge. Transfer experiments indicate that the American species, *Melaphis rhois*, shows an unusual complex life cycle, similar to that known in Chinese melaphidines, with some generations feeding on mosses as alternate host plants. As with the association with sumac, this complex life cycle may have been established in the melaphidine lineage before the southward retreat of sumac from Alaska 48 million years ago. This example suggests that the interactions and life histories shown by modern populations may be determined, in large part, by evolutionary commitments made in the distant past.

DESPITE THE LARGE AMOUNT OF attention paid to possible coevolutionary interactions between herbivorous insects and their host plants, the ages of interactions between specific insect and plant lineages have been estimated in only a few cases (*1, 2*). These ages are difficult to obtain from fossils of damaged plant tissues since the damage must be distinctive enough to be definitely associated with a modern insect group. The ages of life cycle phenomena observed in modern animal species are even more difficult to establish, because these are rarely documented by any fossil evidence. I have used biogeographic evidence to establish the antiquity of an association between an aphid and a plant lineage and of a peculiar complex life cycle.

The aphid subtribe Melaphidina (Homoptera: Aphididae: Pemphiginae: Fordini) consists of four Asian genera and a monospecific American genus (*3, 4*). All known species form galls on sumac species [Anacardiaceae, *Rhus* L., subgenus *Rhus* (*3, 4*)]. These galls are induced by aphid feeding and are inhabited by three generations of parthenogenetic females. Galls are closed, sac-like structures with a structure and composition very different from leaves from which they are derived (*5*). Eclosion of the final winged emigrant generation is synchronized with opening of gall exit slits. Although this level of intricacy suggests that a sumac-Melaphidina association is ancient, more definite evidence concerning the age of the interaction is provided by biogeographic considerations.

The current distribution of the Melaphidina implies that use of sumac was established before the geographic separation of the ancestors of modern hosts in Asia and America. The occurrence of the subgenus *Rhus* in both the Old and New Worlds is attributed to dispersal across the Bering land bridge during or before the early Eocene, an explanation strongly supported by fossil evidence (*6*) (Fig. 1). The vicariance between Asian and American plant lineages resulted when climatic changes pushed plant distributions southward during the Tertiary (*7*). For sumac, this occurred about 48 million years ago, as judged by the distribution of leaf fossils in Alaskan Tertiary floras (*6, 8*). The sumac-Melaphidina association must be

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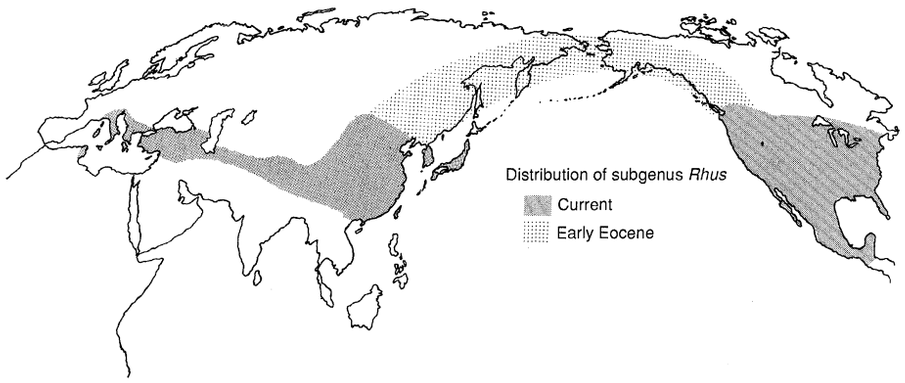

Fig. 1. Approximate current distribution and Eocene dispersal route of the subgenus *Rhus*.

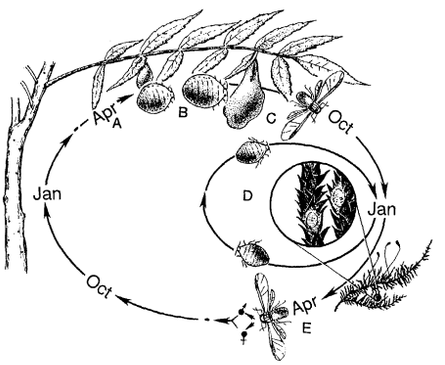

Fig. 2. The life cycle of *Melaphis rhois*. (A) Egg hatches to give rise to fundatrix, whose feeding on a leaflet initiates gall formation. (B) The fundatrix and her wingless daughters develop and produce the migrant generation within the closed gall. (C) Gall slits open and winged migrants eclose and fly to mosses where they deposit nymphs and die. (D) A series of wingless female generations live on mosses, where colonies may persist for more than 1 year. (E) In spring, moss colonies may produce winged migrants that fly to sumac, deposit the sexual generation and die. The dwarf, short-lived sexuals mate and deposit eggs that hatch the following spring to begin another cycle (*18*).

at least this ancient if one accepts that (i) the association with sumac had a single origin within the Melaphidina and (ii) a later, trans-oceanic colonization by the aphids is untenable. The first assumption is virtually certain: all extant melaphidines use sumac and independent acquisitions as the basis for this pattern are implausible. Dispersal across the ocean is also extremely unlikely. The upper estimates of aphid dispersal distances, under optimal wind conditions, are 1300 to 1600 km (*9*). American and Asian sumac populations would have been about 8000 km apart even if they ranged as far north as 55°N on both continents. Furthermore, in Pemphiginae, including Melaphidina, flight is restricted to the short-lived winged generations that move between alternate host-plant taxa.

This estimated minimum age of 48 million years would make this the oldest known continuous association between an insect and a plant lineage. Other estimates of ages of plant-insect associations have been based on fossils of damaged plant tissue rather than on biogeographic evidence (*1, 2*). The oldest such cases for which both plant and insect are generally accepted to correspond to a modern association are fossil lepidopteran mines on oak leaves from middle Miocene deposits in western North America (*1*). The oldest fossil aphid gall reported in the literature is a disputed instance of a pemphigine leaf gall on *Populus* from the Miocene in Europe (*10*).

A similar biogeographic argument can be combined with knowledge of life cycles of modern species on both continents, in order to estimate minimum ages of distinctive life cycle traits. As in numerous other aphid groups, melaphidines show complex life cycles involving alternation between unrelated host plant taxa (*3, 4, 11*). In these heteroecious life cycles, different, morphologically divergent, parthenogenetic generations switch between two discrete sets of hosts that are taxonomically distant and that usually show very different growth forms (*11, 12*). The only melaphidine for which the alternate hosts were previously established is the Chinese species, *Schlechtendalia chinensis* (Bell), which switches from sumac to mosses of the genus *Mnium* (*13, 14*). The alternate hosts of the only North American melaphidine, *Melaphis rhois* (Fitch), have remained a mystery, despite the striking appearance of its galls and its wide distribution on *Rhus typhina* and *Rhus glabra*. Baker's (*15*) description of a *Melaphis* from "mosses" in West Virginia suggested a possible secondary host association. He raised the question of whether the tiny moss-feeding forms, which he called *Melaphis minutus*, might be the alternate generations of the sumac-galling *M. rhois*. He was unable to relocate colonies to attempt confirmatory transfers, and no other reports that I know have established the alternate hosts of *M. rhois*. References to "mosses" as the secondary hosts of *Melaphis* (*16*) are apparently based on the assumption that Baker's suspicion was correct.

I have carried out experiments indicating that mosses are alternate hosts of *M. rhois*. During three different years, migrants collected from *R. glabra* galls were successfully used to initiate long-lived colonies on laboratory cultures of the moss, *Haplocladium microphyllum* (Hedw.) Broth. (*17*). The life cycle of *M. rhois*, inferred from my transfers and Baker's observations, is shown in Fig. 2 (*18*).

This demonstration that heteroecy between sumac and mosses exists in both Asian and American melaphidines implies that, as with the association with sumac, this complex life cycle was established before the Eocene vicariance. The only alternative possibility, that heteroecy to mosses evolved independently on the two continents, can be discounted, since use of mosses by aphids is extremely rare (*19*), and independent acquisitions within Melaphidina are not plausible. Thus, this odd complex life cycle has apparently persisted with little change for at least 48 million years. Such persistence suggests a stability that conflicts with certain theoretical predictions for complex life cycles (*20*) and that contrasts with the more frequent gain and loss of heteroecy characterizing certain other aphid groups (*11, 21*).

Similar combinations of biogeographic and paleobotanical evidence are probably available for a large number of plant-insect associations (*22*). More widespread application of the approach used here may increase our knowledge of the ages of biotic interactions as well as the ages of distinctive life cycle phenomena. The case of *M. rhois* has implications for the study of ecological interactions. If relationships are commonly this old, they have been subjected to selective forces in a wide variety of regimes, including ones very different from the modern habitats in which they are typically studied by ecologists. Further, such antiquity implies that life cycles and host associations may be highly evolutionarily constrained, preventing optimal responses to changing selective forces.

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17. Galls of *M. rhois* were discovered in August 1986 on *R. glabra* at 1990-m elevation in Bear Canyon in the Santa Catalina Mountains, just north of Tucson, Pima County, AZ. Examination of mosses growing on north-facing slopes 50 to 100 m from the sumac revealed tiny aphids occurring singly and encased in white waxy secretions. These were indistinguishable from *M. minutus* of Baker (*15*). The aphids were found only on *H. microphyllum* and not on other intermixed moss species, including *Hypnum pallescens* (Hedw.) P.-Beauv. and *Orthoprochum halli* Sull. & Lesq. *ex* Sull. Four aphid-free cultures of *H. microphyllum* were established by washing the mosses in detergent solution and placing them in paper-lined and covered culture dishes. These were kept in a growth chamber at 20°C with 16L:8D photocycle and watered by wetting the paper with nutrient solution. On 14 September galls were collected from the same stand of *R. glabra*. These galls had slits from which winged migrants were emerging. Some migrants were dissected and their embryos examined to determine whether these could be the winged sexupara morph produced by many Pemphiginae in autumn (though usually not on the galled host plant). The sexuals of Pemphiginae have vestigial mouthparts, whereas these embyros possessed fully developed rostra, indicating that migrants were flying from galls to an alternate host to initiate further parthenogenetic generations. Migrants were transferred to two of the moss cultures in the growth chambers. They deposited nymphs within 8 hours and died within 24 hours. Migrants left in containers with galls did not reproduce or die within the same time interval. Experimental and control (uninfested) *H. microphyllum* cultures were kept in covered dishes in the chamber. After 5 days, small white lumps appeared on the strands of the infested mosses. Microscopic examination revealed that each consisted of a single aphid covered with wax secretions. Feeding was inferred through the presence of droplets of honeydew. The two control cultures lacked aphids, ruling out accidental contamination from aphids present when mosses were collected. Aphids in the laboratory colonies were still reproducing after 26 months and they showed a morphology and feeding habit indistinguishable from that in naturally occuring colonies. Similar transfers from *Rhus* galls to *H. microphyllum* were repeated in September 1987 and in September 1988 with successful establishment of colonies in both years, confirming *H. microphyllum* as the alternate host of *M. rhois*.
18. Baker (*15*) found alatae developing on mosses in April, indicating that the developmental pathway leading to sexuparae (the return migrants to sumac) may be induced by photoperiodic conditions that prevail in spring. This would explain why no alatae developed from my laboratory cultures kept on 16L:8D photocycles. These observations fit with the life cycles recorded for other Melaphidina (*13, 14*) and other Fordini, in which sexuparae fly between alternate host plants in spring [D. Wool, in *Biology of Gall Insects*, T. N. Ananthakrishnan, Ed. (Oxford Univ. Press and IBH Publishing Co., New Delhi, 1984), pp. 11–58; G. Wertheim, *Trans. R. Soc. London* **105**, 79 (1954)]. In *Forda*, the sexually produced eggs are dormant through summer, autumn, and winter. This prolonged dormancy probably occurs in *M. rhois* eggs as well since other aspects of its life cycle resemble those in Fordini and since eggs of other aphid groups have a dormant period.
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23. I thank V. F. Eastop for bringing the Chinese work to my attention, A. Johnson for advice on culturing mosses and for identifying the mosses, J. A. Wolfe for information on the fossil record for *Rhus* in western North America, and D. A. Young for information on *Rhus* taxonomy. G. W. Fernandez, P. M. Mirocha, and M. E. Moran assisted with field collections of *M. rhois*. J. E. Bronstein, M. J. Donoghue, J. A. Glass, R. L. Smith, F. G. Werner, and D. E. Wheeler gave helpful comments on the manuscript. Supported by NSF grant BSR-8806068. This is publication number 7027 of the Arizona Agricultural Experiment Station.

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Introduction of Human DNA into Mouse Eggs by Injection of Dissected Chromosome Fragments

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A procedure has been developed for introducing exogenous DNA into mouse eggs by injection of chromosome fragments. Chromosome fragments were dissected from human metaphase spreads and microinjected into the pronuclei of fertilized mouse eggs. Many of the injected eggs subsequently exhibited normal pre- and postimplantation development. Embryos obtained from eggs injected with centromeric fragments retained human centromeric DNA as demonstrated by in situ hybridization analysis. From eggs injected with noncentromeric fragments, a mouse was obtained whose tail tissue exhibited the presence of human DNA. This procedure should facilitate incorporation of very large (more than 10 megabases) DNA fragments into cells and embryos without the need for cloned sequences.

TRANSGENIC ANIMALS MADE BY DNA injection or retroviral infection (*1, 2*) have been used as powerful model systems for studying gene regulation. Such transformation methods usually result in the insertion of DNA fragments less than 100 kb in size; in cases where it is desirable to introduce a gene cluster or a gene that spans over a great distance, an alternative method may be needed. A possibility that we examined in this study is the direct microinjection of mouse eggs with chromosome fragments containing more than 10 megabases (Mb) of DNA. The feasibility of using this approach is indicated by the previous finding that chromosome fragments can be incorporated into the mammalian karyotype after transfection with calcium phosphate–precipitated chromosomes (*3*). However, the low transformation efficiency of the latter method (*1, 3*) precludes its use for making transgenic animals.

We focused our experiments on the dissection and injection of centromeric fragments, as the persistence of human DNA can be readily detected via the highly repeated centromeric satellite DNA sequences. Centromeric fragments 0.5 to 1.0 μm in size (15 to 30 Mb; estimate based on 3.3×10^9 Mb per haploid genome) were dissected

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Table 1. Maintenance of injected human chromosome fragments in mouse embryos. NA, not applicable.

Fragment injected	Number of experiments	Survived/injected*	Number of embryos		Fragment detection
			Developed/survived†	Embryos recovered‡	
		Preimplantation			
Control	2	21/33 (63%)	12/21 (57%)	NA	NA
Centromere	6	39/90 (43%)	16/39 (41%)	NA	6/12§
		Postimplantation			
Control	2	14/30 (46%)	9/14 (64%)	4 (44%)	NA
Centromere	10	74/135 (54%)	20/74 (27%)	8 (40%)	4/8

*Number of eggs out of the total number still viable 2 to 4 hours after injection. †Number of embryos reaching the morula stage after 4 days in culture. ‡Embryos (12.5 days) recovered from surrogate mothers. §In the six positive embryos, 16 of 82 nuclei and 11 of 89 metaphase spreads exhibited the presence of human satellite DNA.