

Temporal Separation and Speciation in Periodical Cicadas

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Speciation, the set of processes by which two populations of one species become distinct species, is an important topic in evolutionary biology. It is usually impractical to conduct experiments on how new species form, but occasionally the natural history of a species places it in a context that may be thought of as a “natural experiment” with regard to speciation. One such natural experiment involves the periodical cicadas of eastern North America, a group in which populations have become isolated in time and space. Some of these isolated populations appear to have evolved into distinct genetic lineages. A rare life-cycle switching event brought two such lineages into contact in the relatively recent past, and the two lineages are now behaving as distinct species. This natural experiment provides important insights into species differences and the processes that underlie species formation.

Keywords: speciation, secondary contact, reproductive character displacement, allochronic isolation, periodical cicadas

How great a difference is sufficient for two groups of organisms to be considered separate species? Darwin's *On the Origin of Species* ([1859] 1964) showed how complex forms could be changed by natural selection and suggested that local specialization could lead to the emergence of distinct species. After 150 years of research and debate, evolutionary biologists agree that reproductively isolated populations—populations that are unable to exchange genes—may become separate species over time because evolutionary change in one population cannot be communicated to the other. But the details of how populations reach reproductive isolation are still being worked out.

Separated populations have the potential to become distinct species. Both chance and selection cause such populations to diverge. The importance of chance is heightened in small populations, while selection plays an important role when environmental factors differ between the isolated populations. Over time, chance and selection interact in ways that can make it difficult for separated populations to reunite. Such differences may include incompatibilities (behavioral or physiological) in mate location or fertilization, or genetic incompatibilities that prevent hybrids from developing successfully. In other cases, seasonal or distributional differences may prevent adults from reestablishing contact.

So when are separate populations different enough to be considered species? How can we tell whether two populations, isolated for generations by time or space, will remain distinct

or whether they will merge if brought into contact? Such questions probably have no universal answer, but by studying specific examples of closely related populations, we can make inferences about the events involved in species formation. Unfortunately, events critical to understanding speciation processes are almost impossible to observe directly because they generally occur over many generations, at unpredictable times, and in unpredictable locations.

When changes in the distribution or life-cycle timing of a species bring historically isolated populations back into contact, forming a “contact zone” or area of overlap, the results offer unique opportunities to observe events that can help us understand speciation (figure 1). The evolutionary interactions of contact zone populations are complex, and are dampened by factors such as immigration from populations outside the zone. Contact zone populations can affect each other through hybrid sexual interactions; selection on each population is mediated either by the relative quality of purebred versus hybrid offspring or, if no offspring are produced, by the confusion created if the mating signals of the two populations are similar. Sometimes, on coming into contact, populations

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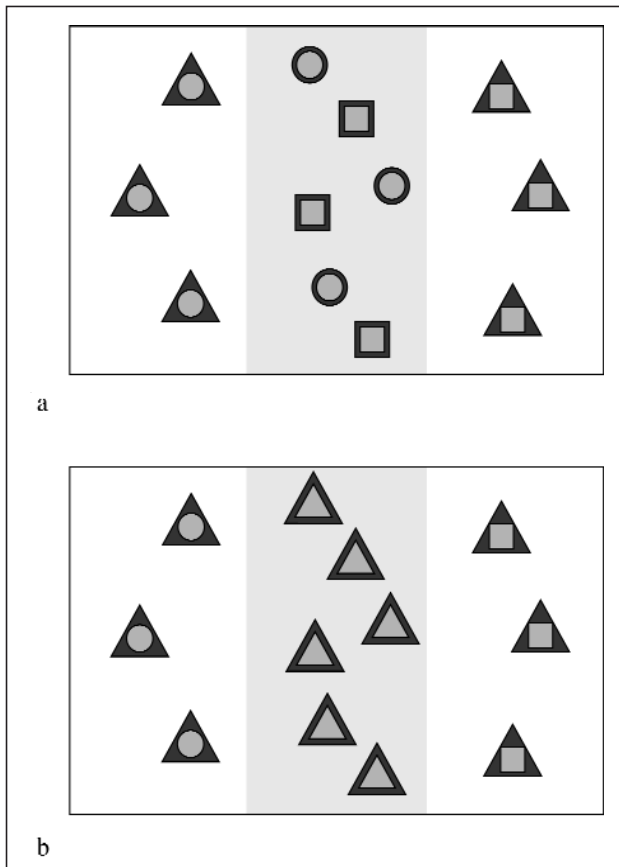


Figure 1. A schematic representation of character displacement. The overlap zone is shaded gray. (a) Two populations undergo overlap reproductive character displacement in the zone of overlap, and their differences are accentuated. (b) Two populations form a hybrid zone, and their differences are reduced.

form a “hybrid zone” that may be stable or may gradually widen as genetic incompatibilities (revealed by hybridization) are removed by natural selection (Jiggins and Mallet 2000). In such cases, gene exchange overwhelms natural selection for assortative mating (mating with one’s own type; e.g., Hewitt 1993, Butlin 1998, Jiggins and Mallet 2000). Alternatively, populations coming into contact can undergo reproductive character displacement, in which selection reduces the possibility of cross-species sexual interactions by accentuating differences in sexual signals (Brown and Wilson 1956, Howard 1993). Under these circumstances, selection for assortative mating prevails, decreasing the possibilities for hybridization. Finally, formerly separated populations could be so differentiated that, after coming into contact, they coexist without hybridizing at all. Which of these outcomes occurs is determined by factors such as the sexual attractiveness of the members of each population to members of the other and the biological possibility of the formation and successful reproduction of hybrids. Generally, the greater the differences between populations upon contact, the greater the likelihood they will retain their distinctiveness.

Taking advantage of natural experiments afforded by secondary contact requires luck. Hybridizing forms may quickly merge into one uniform population, and patterns of character displacement may be rapidly lost as species’ ranges shift. Our research has uncovered a case of recent contact between two genetic lineages of periodical cicada (*Magicicada* spp.). This contact allows a unique look at interactions between lineages that probably were separated less than 2 million years ago.

Periodical cicada biology

Periodical cicadas (*Magicicada* spp.; figure 2) are among the world’s most enigmatic insects. They are found only in the United States, east of the Great Plains. Their population densities are astonishing, approaching 1 million per acre (Dybas and Davis 1962). Juveniles live underground in meticulously constructed tunnels and feed by sucking root fluids for 13 or 17 years. In the spring of their final year, nymphs leave their burrows and complete their fifth and final molt. If conditions are appropriate, nearly the entire population may emerge in the space of only a few warm evenings. Adult periodical cicadas live four to six weeks; their black bodies, large wings, red eyes, and charismatic behaviors are unmistakable. Males possess, on their abdomens, specialized sound-producing organs called *tymbals*, which they use to produce loud rhythmic or musical acoustic signals to attract mates. Fully mature adult male *Magicicada* form dense singing aggregations in which each male cicada makes a small number of signals before flying to another calling perch. Females visit these choruses to mate; on hearing an individual male’s signal, a fully mature, sexually receptive female flicks her wings, producing a subtle response signal (Cooley and Marshall 2001). The male reacts by making stereotypical courtship sounds and movements. Females lay their eggs shortly after mating, and most mate only once. (Periodical cicada biology is reviewed in Alexander and Moore 1962, Simon 1988, and Williams and Simon 1995.)

Periodical cicadas in different regions are not synchronized, so an emergence occurs somewhere almost every year. Emergences tend to be regional, and populations emerging together on a given 13- or 17-year schedule are collectively termed a *brood*. By convention, broods are designated by roman numerals reflecting their order of appearance (Marlatt 1907; see table 1). There are twelve known 17-year broods (I–X, XIII, and XIV) and three 13-year broods (XIX, XXII, and XXIII). Most broods were probably formed since the last glacial maximum (approximately 18,000 years ago), because they occupy regions in eastern North America that were inhospitable to them during glaciation. The broods overlap only slightly and have complex geographical relationships; their spatial relationships suggest that broods are formed when climatic processes cause subpopulations to emerge in unexpected years and adopt a new emergence schedule (Alexander and Moore 1962, Lloyd and Dybas 1966, Lloyd and White 1976).



Figure 2. Periodical cicadas. Clockwise from top left, *Magicicada septendecim* mating pair; *Magicicada* nymph emerging from burrow after 17 years; *M. septendecim* on thorn bush; and female *M. septendecim* laying eggs. Photographs: © Lang Elliott, NatureSound Studio, Ithaca, New York; www.naturesound.com.

Superimposed on the complex biogeography of *Magicicada* broods are the relationships of the seven *Magicicada* species. Three distinct species with 17-year life cycles coexist in northern and Great Plains states (*M. septendecim*, *M. cassini*, and *M. septendecula*). In the Midwest and South, four 13-year species have been described (*M. tredecim*, *M. neotredecim*, *M. tredecassini*, and *M. tredecula*). The seven species fall into three natural groups of close relatives; these three morphologically and behaviorally distinct groups are generally abbreviated *decim*, *cassini*, and *decula*. Within each group, the species are extremely similar in appearance, behavior, male song, and genetics. Each species appears most closely related to one with the other life cycle, suggesting that *Magicicada* species form in part by permanent life cycle change (figure 3). With few exceptions, each brood of a given life cycle contains species belonging to all three species groups, although some populations of each brood are lacking one or more species. Unlike the modern broods, most species very likely predate the last glacial cycle; thus, biogeographic clues as to their origins have been erased by postglacial range changes (Marshall and Cooley 2000, Simon et al. 2000, Cooley et al. 2001).

One of the most exciting recent events in the study of periodical cicadas was the discovery of the seventh periodical species, not previously distinguished from *M. tredecim*, and now named *M. neotredecim*. *Magicicada neotredecim* probably arose during the Holocene (after the last glacial maximum) when some members of the 17-year species *M. septendecim* underwent a permanent life cycle change and became 13-year cicadas. The recency of this speciation event means that biogeographical clues concerning its interactions with other periodical cicada species have been preserved (Marshall and Cooley 2000, Simon et al. 2000, Cooley et al. 2001).

The discovery of *M. neotredecim*

In most cases, closely related 13- and 17-year species are distinguishable only by life-cycle length. However, as early as 1962, *M. tredecim* was found to contain two forms, one with more orange on the ventral surface of the abdomen and the other with more black (Alexander and Moore 1962). By the late 1980s, this color difference was found to correlate with a sharp genetic break between northern and southern populations (Martin and Simon 1988, 1990a, 1990b). The north-

Table 1. The current periodical cicada brood classification, devised by Marlatt (1907). Starting in 1893, Marlatt numbered 17-year brood year classes sequentially from I to XVII, and 13-year brood year classes from XVIII to XXX. Currently, there are only twelve recognized 17-year broods and three recognized 13-year broods (Simon 1988).

| Brood | Life cycle | Emergence | | Species | Region |
|-------|------------|-----------|------|---|--|
| | | Previous | Next | | |
| I | 17 | 1995 | 2012 | <i>septendecim, cassini, septendecula</i> | VA, WV |
| II | 17 | 1996 | 2013 | <i>septendecim, cassini, septendecula</i> | CT, MD, NC, NJ, NY, PA, VA |
| III | 17 | 1997 | 2014 | <i>septendecim, cassini, septendecula</i> | IA, IL, MO |
| IV | 17 | 1998 | 2015 | <i>septendecim, cassini, septendecula</i> | IA, KS, MO, NE, OK, TX |
| V | 17 | 1999 | 2016 | <i>septendecim, cassini, septendecula</i> | MD, OH, PA, VA, WV |
| VI | 17 | 2000 | 2017 | <i>septendecim, cassini, septendecula</i> | GA, NC, SC |
| VII | 17 | 2001 | 2018 | <i>septendecim</i> | NY |
| VIII | 17 | 2002 | 2019 | <i>septendecim, cassini, septendecula</i> | OH, PA, WV |
| IX | 17 | 1986 | 2003 | <i>septendecim, cassini, septendecula</i> | NC, VA, WV |
| X | 17 | 1987 | 2004 | <i>septendecim, cassini, septendecula</i> | DE, GA, IL, IN, KY, MD, MI, NC, NJ, NY, OH, PA, TN, VA, WV |
| XIII | 17 | 1990 | 2007 | <i>septendecim, cassini, septendecula</i> | IA, IL, IN, MI, WI |
| XIV | 17 | 1991 | 2008 | <i>septendecim, cassini, septendecula</i> | KY, GA, IN, MA, MD, NC, NJ, NY, OH, PA, TN, VA, WV |
| XIX | 13 | 1998 | 2011 | <i>tredecim, neotredecim, tredecassini, tredecula</i> | AL, AR, GA, IN, IL, KY, LA, MD, MO, MS, NC, OK, SC, TN, VA |
| XXII | 13 | 2001 | 2014 | <i>tredecim, tredecassini, tredecula</i> | LA, MS |
| XXIII | 13 | 2002 | 2015 | <i>tredecim, neotredecim, tredecassini, tredecula</i> | AR, IL, IN, KY, LA, MO, MS, TN |

ern, darker form was found to be genetically and morphologically similar to the 17-year species *M. septendecim*, while the southern, more orange form was morphologically and genetically distinct. The two kinds of 13-year *decim* were found to overlap only slightly in a narrow region stretching from Arkansas to southern Indiana. A recently discovered song difference suggested that these two types of 13-year *decim* were separate species (Marshall and Cooley 2000). Within the narrow zone of overlap, northern-type males produce high-pitched calling songs (1.71 kilohertz [kHz], or approximately the pitch of A two octaves above middle C), while southern-type males have low-pitched calling songs (1.11 kHz, or approximately the pitch of C-sharp two octaves above middle C; figure 4). Courtship songs and female pitch preferences of the two species show similar shifts, so the two types appear to mate assortatively, with no genetic, morphological, or behavioral evidence of hybridization (Simon et al. 2000, Cooley et al. 2001).

Because of these discoveries, the northern form of 13-year cicada was designated as a separate species, *M. neotredecim*, while the southern form retained the name *M. tredecim*. The genetic, morphological, and behavioral evidence suggests that *M. neotredecim* was formed from a population of *M. septendecim* that switched from a 17-year life cycle to a 13-year one.

Why was this song difference discovered now and not earlier? Earlier biologists missed this striking difference because the disparity in pitch is much reduced outside the contact zone. In 1998, purely by chance, the site chosen for behavioral studies was within the contact zone.

A natural experiment

The ranges of *M. septendecim* (a 17-year species) and *M. tredecim* (a 13-year species) overlap only minimally. Even where these species do overlap, because of their life-cycle differ-

ence, they emerge simultaneously only once every 221 years. The formation of a new 13-year species (*M. neotreddecim*), synchronized with an existing 13-year species (*M. treddecim*), created new opportunities for contact between the previously separated *M. septendecim*/*M. neotreddecim* lineage and the *M. treddecim* lineage. What were the results of this natural experiment? Instead of forming a hybrid zone, the interacting populations appear to have maintained their distinctiveness, and the accentuation of these species' signal differences fits the pattern of reproductive character displacement. Where both species are present, *M. neotreddecim* male calling songs are highest in pitch (1.7 kHz) and female mating preferences match; such high-pitched songs are found nowhere else. Away from the overlap zone, where *M. treddecim* is absent, *M. neotreddecim* male pitch and female pitch preference are considerably lower, ranging from 1.3 to 1.5 kHz. A weaker reciprocal pattern of song and preference variation may exist in *M. treddecim* (Marshall and Cooley 2000).

Can temporal differences be sufficient to separate species?

There has been some debate as to whether allochronically separated species should be considered valid species, because life cycle differences are often plastic (not genetically fixed) and may not fully isolate populations. Some *Magicicada* species differ only in life-cycle length, and Darwin himself noted in his correspondence (in Riley 1869) that without more information there was insufficient evidence to equate life-cycle differences with species differences in *Magicicada*. Since Darwin's time, however, we have come to a better understanding of *Magicicada* life cycles. Life-cycle stability after transplantation into different habitats (Lloyd 1987) and historical stability of the border between 13- and 17-year species (Simon 1988) suggest that *Magicicada* life cycles are not particularly plastic and that they are not cued by short-term climatic fluctuations. The best demonstration of the validity of designating at least some of the *Magicicada* life-cycle types as separate species comes from the natural experiment involving *M. neotreddecim* and *M. treddecim*. The pattern of reproductive character displacement suggests that *M. neotreddecim* and *M. treddecim*, and by extension *M. septendecim* and *M. treddecim*, are separate species.

Character displacement and species. Some models of speciation, such as the reinforcement model, invoke processes similar to reproductive character displacement. Under this model, two populations of the same species become isolated and subsequently diverge. When they come into contact again, selection against hybrids "reinforces" or accentuates their differences, resulting in two separate "sister species." Because genetic exchange has an autocatalytic quality that tends to erode rather than enhance genetic barriers, such reproductive character displacement is likely only when opportunities for genetic exchange are limited (Butlin 1987, 1989, Rice and Hostert 1993, Noor 1999). Critics of the reinforcement model suggest that many potential incipient species often retain

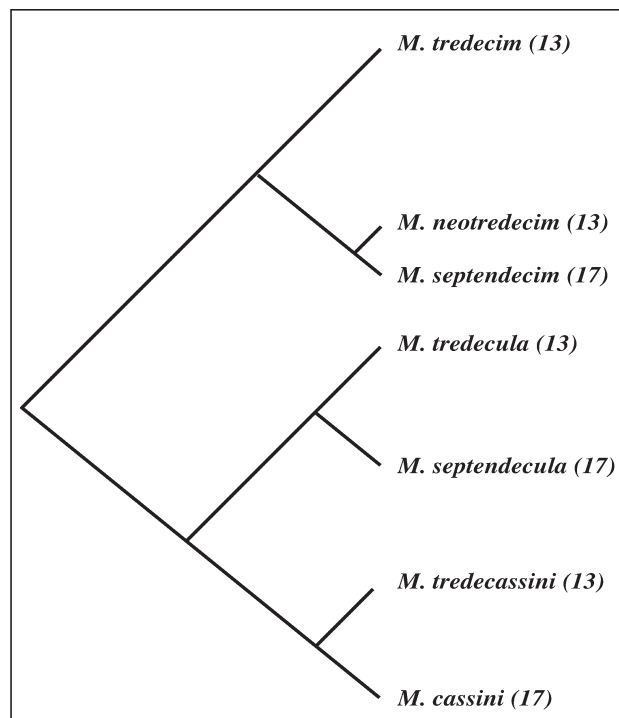


Figure 3. The relationships of the seven periodical cicada species (*Magicicada* spp.), with life cycles in parentheses. Each species is most closely related to another with a different life cycle.

interbreeding capabilities sufficient to make character displacement implausible.

Magicicada does not fit the typical model of speciation by reinforcement, because all available evidence indicates that the two species involved in displacement (*M. treddecim* and *M. neotreddecim*) are not each other's closest relatives, and because the event that formed *M. neotreddecim* was an allochronic speciation event (a life-cycle change) that was probably independent of any displacement. This example illustrates an important point: Reproductive character displacement is sometimes less a part of the processes responsible for permanently separating lineages than a possible outcome after such separation has been achieved (Remington 1968). Yet the reproductive character displacement in *Magicicada* involves many of the same processes invoked by the reinforcement model, and leads to many similar kinds of questions about past and present genetic exchange between the *M. treddecim* and *M. septendecim*/*M. neotreddecim* genetic lineages. Since *M. neotreddecim* did not amalgamate with *M. treddecim*, how much or how little genetic exchange was there at contact?

Looking at the past. There is every indication that the reproductive barrier between *M. treddecim* and *M. neotreddecim* is relatively impermeable; we have found few phenotypically intermediate individuals or putative hybrids. This result is exactly what we expect as an outcome of reproductive character displacement. But were these two species always so thoroughly isolated? Is the pattern of reproductive character

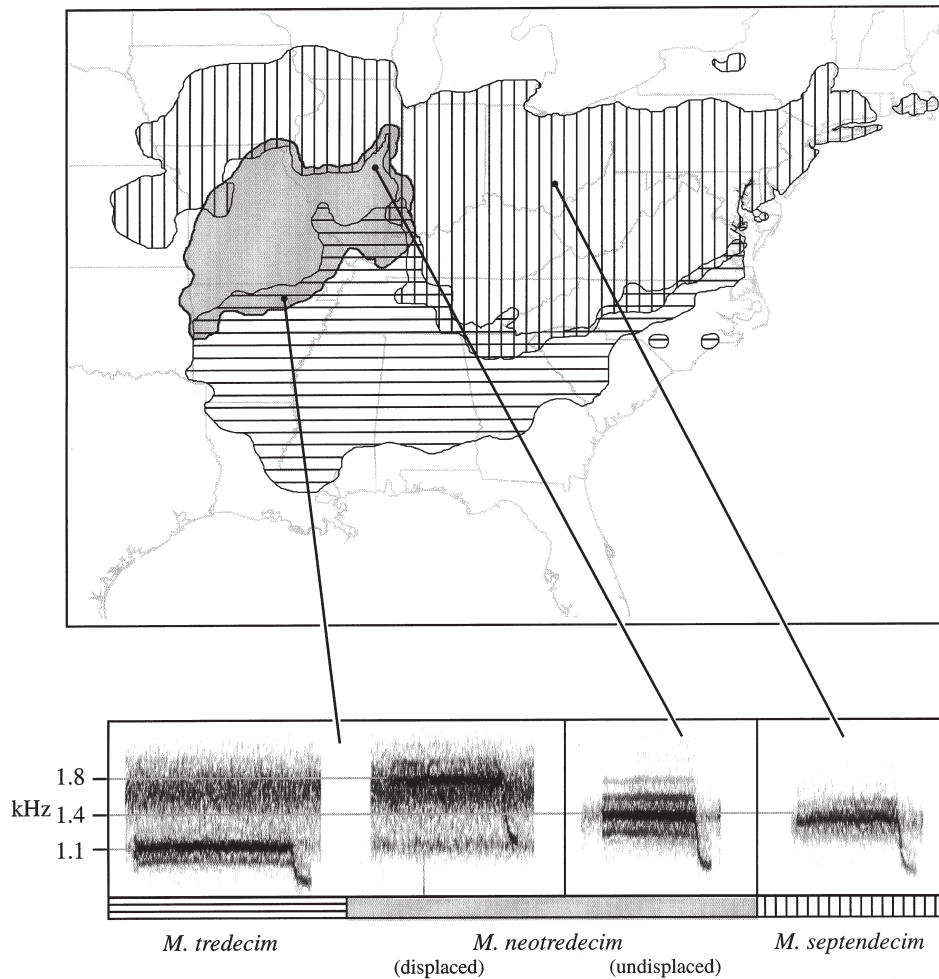


Figure 4. The distribution of three *Magicicada* species: *M. septendecim* (vertical lines), *M. tredecim* (horizontal lines), and *M. neotreddecim* (shaded). These three species overlap only slightly. Below are appended sonograms, or graphs of song pitch (kilohertz [kHz]) versus time, for typical *M. septendecim* and *M. tredecim* and for displaced and undisplaced *M. neotreddecim*.

displacement best explained by selection to avoid production of low-quality hybrid offspring, or by selection that resulted from interactions involving mating signals? Because reproductive character displacement changes the way species interact, it tends to obscure clues about its selective basis (Pritchard and Schluter 2000). Although biologists cannot recreate the past, two approaches, comparative studies and “fossil” evidence, can illuminate the past.

Periodical cicada species provide unique opportunities for comparative studies, because present-day *M. septendecim* are probably extremely similar, genetically and behaviorally, to the long-gone ancestors of today’s character-displaced *M. neotreddecim*. Thus, interactions between the 13-year species *M. tredecim* and the 17-year species *M. septendecim* could resemble the initial interactions between *M. tredecim* and *M. neotreddecim*. Although adjacent 13- and 17-year populations overlap only minimally and emerge simultaneously just once every 221 years, modern transportation allows us to bring these species together experimentally even if their populations are not in close proximity. In addition, playbacks of recorded and

simulated male calls allow us to expose members of one species to behaviors typical of the other. Continued biogeographic monitoring is another approach; hybrids between *M. septendecim* and *M. tredecim* could have unusual, intermediate life cycles. By monitoring selected locations along the boundary between these two species, we can detect unexpected emergences suggestive of hybridization (as in Lloyd et al. 1983).

What sorts of fossils are left by past genetic exchange? Genetic exchange erodes sharp genetic divisions. Thus, “fossil” evidence for past genetic exchange would be similar to evidence for present-day hybridization—genetic markers that are characteristic of one population would occasionally be found in the other. If hybridization occurred to any appreciable extent in the past, then individuals with an occasional genetic marker characteristic of the other species could still be detectable at present by various genetic fingerprinting methods. At present, our data show an exact species specificity of genetic markers, such as mitochondrial DNA haplotype, and calling-song pitch or pitch preference. These results are

consistent with a hypothesis that selection arising from the consequences of infertile hybrid matings best explains the observed pattern of reproductive character displacement.

Conclusions

How distinct are species? Although it would be misleading to suggest that some threshold level of genetic difference prescribes designation as separate species, there is still some heuristic value in probing the lower limits of species' genetic distinctiveness. Theoretically, a single nucleotide difference could result in complete reproductive isolation; at the same time, two populations could differ by substantial numbers of nucleotides without impediment to full genetic exchange. The mitochondrial sequence difference between the *M. tredecim* and *M. neotredecim*/*M. septendecim* lineages is estimated to have been approximately 2.5% when the *M. tredecim*/*M. neotredecim* contact zone formed. Studies of the mitochondrial DNA of other insect species suggests that this genetic distance correlates roughly with 1 million years of separation (Martin and Simon 1990a). In *Magicicada*, this magnitude of genetic difference appears to be associated with permanent separation, because *M. tredecim* and *M. neotredecim* underwent reproductive character displacement instead of forming a hybrid zone. Even though no broad generalization from these results is warranted, *Magicicada* do provide an important new case study in the genetics of reproductive isolation (Bridle and Ritchie 2001).

More details of periodical cicada biology and our current studies of periodical cicada genetics can be found at www.eeb.uconn.edu/collections/cicadacentral/ and http://insects.ummz.lsa.umich.edu/fauna/michigan_cicadas/index.html.

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