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GENETIC EVIDENCE FOR ASSORTATIVE MATING BETWEEN 13-YEAR CICADAS AND SYMPATRIC ''17-YEAR CICADAS WITH 13-YEAR LIFE CYCLES'' PROVIDES SUPPORT FOR ALLOCHRONIC SPECIATION

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Abstract.—Thirteen-year cicadas of brood XIX from northern Arkansas, Missouri, and southern Illinois (lineage A) are known to be genetically different at two marker loci (mitochondrial DNA and abdominal color) from 13-year cicadas to the south (lineage B) that emerge in the same year. Because 17-year cicadas from all broods (year classes) are indistinguishable from lineage A at these two marker loci, previous workers suggested that the lineage A cicadas of 13-year brood XIX were derived from 17-year cicadas by life-cycle switching (allochrony). Data presented here show that, over the same northern geographic range, lineage A is also present in 13-year cicadas belonging to brood XXIII (which always emerges four years later than brood XIX). Detailed sampling along the putative life-cycle-switching boundary in 13-year brood XXIII revealed a previously unsuspected broad zone of overlap where populations contained individuals of both lineages A and B. Despite this sympatry, and previous reports of a lack of behavioral barriers to interbreeding, a strong correlation between mitochondrial haplotype and abdominal color suggests that assortative mating has taken place. Lineage A 13-year cicadas from both broods XIX and XXIII are only found within a gap in the spatial distribution of 17-year cicadas. This, in combination with the lack of differentiation between lineage A 13- and 17-year cicadas at the marker loci and new behavioral data for 13-year brood XIX, suggests a recent derivation of all northern 13-year cicadas from the 17-year cicadas via life-cycle switching. We discuss the implications of these allochronic shifts for speciation.

Key words.—Allochronic speciation, assortative mating, color polymorphism, hybridization, *Magicicada,* risk spreading, secondary contact.

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Periodical cicadas have attracted the attention of many biologists interested in life- history evolution. Their work has focused on the evolution of the long life cycles (Heliovaara et al. 1994), the evolution of prime-numbered life cycles (Lloyd and Dybas 1966; Yoshimura 1997; Cox and Carleton 1998), the evolution of synchronized periodicity (Bulmer 1977; May 1979), and the occurrence of life-cycle shifts (Lloyd and White 1976; Simon and Lloyd 1982; Kritsky 1988, 1992; Martin and Simon 1990a; Williams and Simon 1995). This paper focuses on the importance of life-cycle shifts for species formation. Periodical cicada life history is complex and an introduction to it is essential for any discussion concerning these species.

Periodical cicadas live only in the United States east of the Great Plains. Thirteen-year cicadas are found mainly in the southern parts of the distribution and 17-year cicadas mainly in the north (Marlatt 1907; Simon 1988). Not all periodical cicadas emerge in the same year; rather, their geographic range is a mosaic of spatially contiguous populations comprising year classes known as broods. There are 17 possible years during which different broods of 17-year cicadas could emerge (numbered sequentially from I to XVII); however, only 12 of these year classes currently exist (I–X, XIII, and XIV). Of the missing broods, some (e.g., XI) are known to have gone extinct, while others may never have existed (e.g., XVI and XVII) or are discounted because they are thought to be composed entirely of stragglers from larger broods emerging in the wrong year (e.g., XV). Analogously, although there are 13 possible years during which different 13-year broods could emerge (numbered sequentially from XVIII to XXX), there are only three well-documented recurrent 13-year broods. Of the three, two are large (XIX and XXIII) and one is small (XXII). The brood numbers we currently use were designated by Marlatt (1898) who chose 1893, arbitrarily, for 17-year brood I and 13-year brood XVIII. Although brood identity is based solely on year of emergence, most populations of a life cycle appearing in the same year are thought to be evolutionarily related (Simon 1983; for exceptions, see Simon and Lloyd 1982). The larger number of 17-year broods compared to 13-year broods may be related to increased frequency of localized life-cycle shifts in northern populations associated with Pleistocene glaciation (Williams and Simon 1995).

Periodical cicadas have been dubbed ''predator foolhardy'' (Beamer 1931; Lloyd and Dybas 1966) due to their ease of capture compared to other cicadas. Their strategy for survival is predator satiation rather than predator avoidance (Karban 1982; Williams et al. 1993). Alexander and Moore (1962) named six species of periodical cicadas comprising three morphologically and behaviorally distinct forms, each of which had a 13-/17-year virtually indistinguishable sibling-species pair: *Magicicada tredecim/septendecim; M. tredecassini/cassini*; and *M. tredecula/septendecula.* The three distinct forms are commonly referred to as ''decim,'' ''cassini,'' and ''decula'' for convenience. Most broods contain all three morphologically distinct species, which co-occur throughout most of their ranges. The three are behaviorally isolated in the field by their distinctive species-specific calls, but interbreeding can occur if they are confined in small cages (Alexander and Moore 1962) and viable young can be produced (White 1973). Matings between the 13- and 17-year species of each pair can also be induced and result in viable nymphs

(Lloyd and Dybas 1966). This paper concerns only the decim species pair.

Martin and Simon (1988; 1990b) studied the decim species of 13-year brood XIX and found that it comprised a northern and a southern genetic lineage identifiable by mitochondrial haplotype, abdominal color, and allele frequency at the phosphoglucomutase (*Pgm*) allozyme locus. The northern lineage possessed a mitochondrial haplotype, A, which differed by an estimated 2.6% of nucleotide sites from the southern lineage, B, had significantly blacker markings on the orange abdominal sternites; and had populations characterized by a lower frequency of the *Pgm* A allele. The northern lineage was indistinguishable at these three marker loci from 17-year cicadas sampled over a broad geographic area (Martin and Simon 1988, 1990b; Simon et al. 1993). The geographic boundary between the northern and southern lineages of brood XIX stretched from northern Arkansas to southern Illinois and Indiana (Fig. 1A). There was an abrupt transition between the two lineages at this boundary and no mixed populations were detected (Fig. 2A). These data, in combination with the fact that this northern 13-year lineage fits neatly into a hole in the distribution of the 17-year cicadas (Fig. 1B), suggested to Martin and Simon that the northern 13-year cicadas were derived from 17-year cicadas. The lack of genetic differentiation between the northern lineage and the 17-year cicadas and the fact that much of their geographic range was glaciated 20,000 years ago suggested a recent origin. The derivation of one periodical cicada brood from another by four-year changes in the life cycle was not a new idea. It was first suggested by Lloyd and Dybas (1966) and discussed in various contexts by many authors since that time (White and Lloyd 1975; Lloyd and White 1976; Simon et al. 1981; Simon and Lloyd 1982; Kritsky 1988, 1992). Four-year acceleration events were viewed by these authors as important in early postglacial times, when periodical cicada populations were likely larger and more continuous. In contrast, the documented four-year acceleration events of the 20th century, were for the most part viewed as evolutionary dead ends. None of these previous authors ever suspected that the large 13-year broods were made up of two different genetic lineages. Because the discovery of the two 13-year lineages was unexpected, a thorough search of the potential contact zone was not undertaken. Following the discovery of the two lineages, Martin and Simon proposed that secondary contact, if it existed, would result in random mating and gene flow and the eventual obliteration of any genetic differences that may have evolved in isolation (Martin and Simon 1988, 1990b; Williams and Simon 1995). This prediction was based on the fact that no behavioral differences between 13- and 17-year sibling species of periodical cicadas had ever been found (Alexander and Moore 1962; Lloyd and Dybas 1966).

The existence of the two lineages immediately raised the question of whether more detailed sampling along the life-cycle switching boundary in 13-year brood XIX might reveal genetically mixed populations and whether this unusual life-cycle-switching phenomenon would be found in the only other year class of 13-year cicadas that occupies this same large geographic area, brood XXIII (Fig. 1C). Here, we address both of these questions. Our data analyses, completed in 1997, support the conclusions that a similar life-cycle-switching event is evident in brood XXIII and that there are populations along the life-cycle-switching boundary that contain individuals of both lineages. However, contrary to the expectations of Martin and Simon (1988, 1990b), our data suggest that random mating has not occurred. Our data also suggest that more detailed sampling of Martin and Simon's (1988; 1990b) 13-year brood XIX when it next emerged in 1998, would reveal both lineages in populations along the same life-cycle-switching boundary.

Marshall and Cooley (2000) studied 13-year-cicada brood XIX in 1998 and produced many findings relevant to those presented here: (1) using abdominal color as a proxy for the mitochondrial lineages, they found song pitch to differ between southern lineage B 13-year cicadas and northern lineage A 13-year cicadas; (2) this song pitch difference was recognized by females of the two lineages (identified by abdomen color); (3) females preferred the song type of their own lineage; and (4) along the life-cycle-switching boundary, populations were found containing both lineages (as identified by color and song). An exciting component of Marshall and Cooley's study was the discovery of strong reproductive character displacement in the mixed-lineage populations. Following the presentation of our brood XXIII data below, we discuss the implications of our findings and those of Marshall and Cooley for the formation of broods and species of periodical cicadas.

Materials and Method

Samples of 13-year cicada brood XXIII were collected from 45 populations throughout the range of their distribution and frozen on dry ice to be used in a variety of studies. These records and others can be viewed and searched via a link through the following website: www.eeb.uconn.edu/faculty/simon/simon.htm. Specimens were stored at -70°C. From these samples the mtDNAs of 216 individuals representing 13 populations (average 16.6 individuals per population; range = 8–29) were haplotyped (Table 1). Extractions were carried out as published in Martin and Simon (1988) and Phillips and Simon (1995). Mitochondrial DNA haplotypes were identified as in our previous work, using polymerase chain reaction/restriction-fragment-length polymorphism analysis (PCR/RFLP) of the mitochondrial COII, A6, A8, and COIII genes (Simon et al. 1993) or heteroduplex analysis (HDA; Tang et al. 1998) of a portion of the mitochondrial large subunit rRNA (16S) gene.

Primers for the amplification of the COII-A6-A8-COIII region were C2-J-3696 and C3-N-5460, and primers for the amplification of the 16S gene were LR-J-12887 and LR-N-13398 (Simon et al. 1994). PCR/RFLP involved cutting the amplified product with four restriction enzymes—MspI, BspHI, BglII, and DraI—which each produced products diagnostic for 13- and 17-year decim species. Heteroduplex analysis employed amplified fragments of the mitochondrial 16S rRNA gene of the two decim lineages A and B (''test samples'') and a ''driver DNA'' cloned from *M. septendecula.* Each test sample and the driver DNA were mixed and hybridized to form heteroduplex products, which were separated by acrylamide gel electrophoresis. Sequence variation among test samples produced differences in the number of

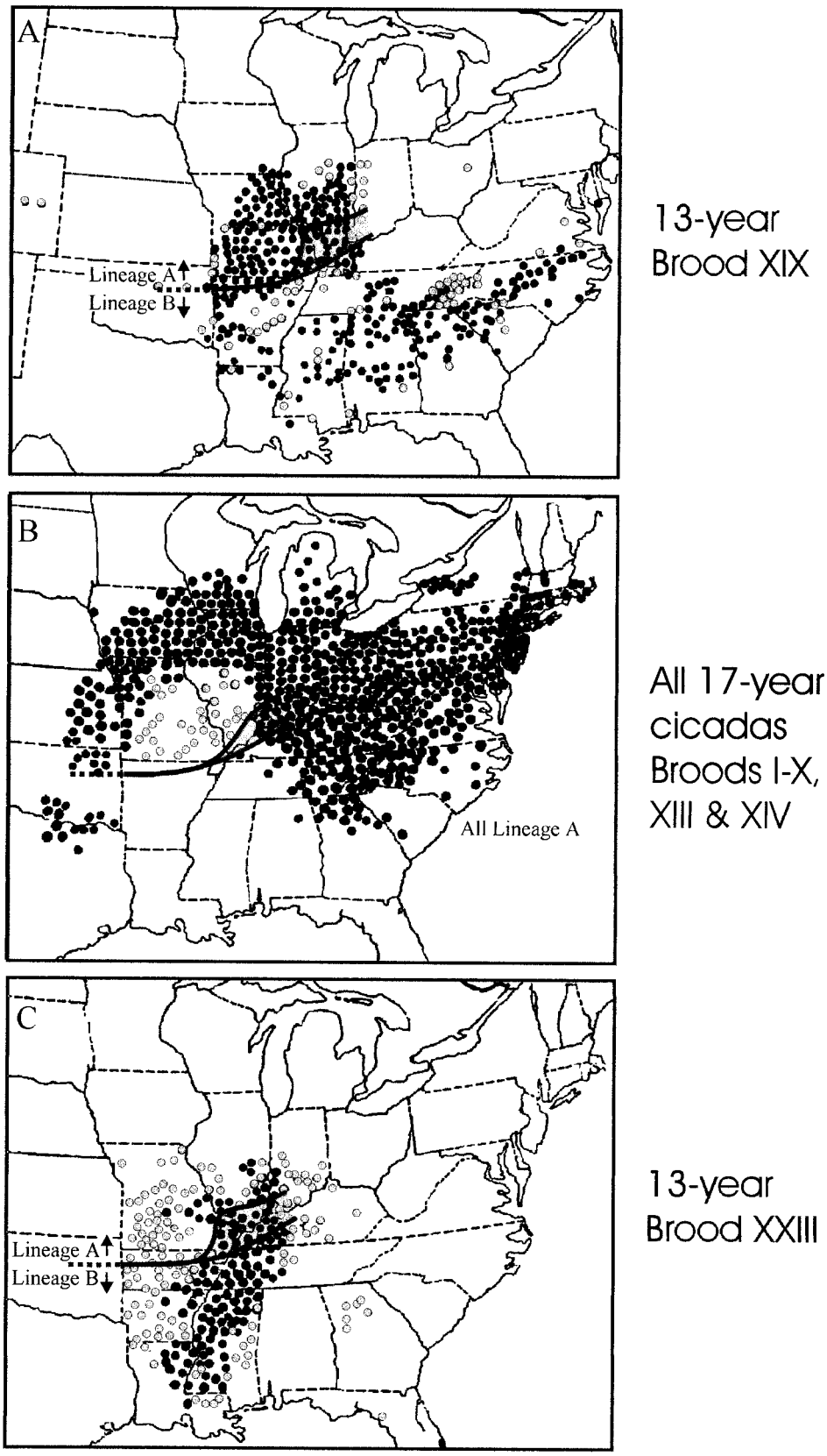

FIG. 1. Distributions of periodical cicadas redrawn and modified (Simon 1979, 1988). Black circles represent counties from which periodical cicadas have been reported. Gray circles indicate areas in which periodical cicadas are believed to have gone extinct or records of doubtful validity (''straggler'' cicadas from other broods appearing one or four years early or late). Approximate boundary lines

unpaired and mismatched bases in DNA-heteroduplex products, resulting in distinct gel mobility shifts. Under the conditions outlined elsewhere (Tang and Unnasch 1995), a single base-pair change could be detected by this technique. In addition, at least four cloned examples with identical sequences were obtained for each decim HDA haplotype to represent either lineage A or lineage B sequences. These sequences were deposited in Genbank along with sequences of *M. septendecula* and *M. cassini* (numbers U41629–U41632). Table 1 gives locality names, sample sizes, exact percentages of each mitochondrial haplotype, and corresponding mean abdominal color scores. Some individuals were typed using PCR/RFLP only and other using HDA only. Ten individuals were typed using both methods to verify correspondence of the two techniques.

Abdominal sternite colors were scored as previously described (Martin and Simon 1988) by assigning a number between zero and four to specimens whose abdomens were dissected down the back midline, cleaned, flattened, dried, and compared to four similarly prepared abdomens chosen as representatives of each color state. Color photographs of the representative abdomens can be viewed on our website ''Magicicada Central,'' a subsite of Cicada Central, which can be accessed via a link from www.eeb.uconn.edu/faculty/simon/simon.htm. Abdomens were presented to the single scorer (CS) in a randomized, blind fashion and scored as continuous decimal numbers rather than integers. Total color scores, reported in Table 1, are calculated by multiplying the mean color score by a constant, 20, to make each comparable to published values (Martin and Simon 1988). Populations containing only lineage A mitochondrial haplotypes were tested for differences in median color score from populations containing only lineage B using the Mann-Whitney *U*-test (Sokal and Rohlf 1969). In addition, for all populations containing both lineages, median color scores of individuals of mitochondrial lineage A were compared to median color scores of individuals of mitochondrial lineage B using this same test.

Results

Our genetic survey of 13-year decim from brood XXIII supports the hypothesis that similar, large-scale, life-cycle switching has occurred in the same geographic area as in brood XIX (Fig. 2, Table 1). In this case as in brood XIX, 13-year cicadas in the north were identical in mitochondrial haplotype (lineage A) to the 17-year *M. septendecim* sampled previously (Martin and Simon 1988, 1990b; Simon et al. 1993). A Mann-Whitney *U*-test (Sokal and Rohlf 1969) established that individuals of 13-year brood XXIII from pure lineage A populations were significantly different in abdominal coloration from individuals of 13-year brood XXIII from pure lineage B populations ($U = 916.5$, $z = -7.24$, $P < 0.0001$).

In addition, intense sampling along the suspected life-cycle-switching boundary revealed a 100-km-wide zone of overlap between the two lineages where populations were found with both mitochondrial haplotypes (Fig. 2B, Table 1). In these intermixed populations, lineage B always predominated and there was no spatial segregation by mitochondrial haplotype. We tested the 62 individuals from mixed populations (12 lineage A and 50 lineage B; Table 1) for differences in median color score. Despite the fact that these cicadas were reproducing side by side and despite previous suggestions of a lack of any behavioral barrier to mating (Alexander and Moore 1962; Lloyd and Dybas 1966), our data suggested nonrandom, or ''assortative,'' mating. In these mixed populations, we found the same association between the biparentally inherited abdominal color and maternally inherited mitochondrial lineages that was found in unmixed populations. Individuals possessing mitochondrial lineage A were significantly darker in median abdominal sternite color than individuals possessing mitochondrial lineage B ($U = 52$, $z = -4.44$, $P < 0.05$).

Abdominal color is a very good but not an absolute discriminator of the two lineages. In pure 17-year cicada populations (all lineage A), far removed from any 13-year cicadas, there are occasional individuals with color scores around 3.5 (i.e., more orange than expected; A.F. Paradis and C.Simon, unpubl. data). Our brood XXIII samples contain a few odd-colored individuals—two lineage A individuals with a color score of 3.5 and 3.8, and four lineage B individuals with color scores of 2.0, 2.2, 2.5, and 2.5—which could represent such variation or could result from occasional interbreeding.

Discussion

Five important new findings have emerged from this study: (1) In brood XXIII, 13-year cicadas occur as two lineages, A (northern) and B (southern), that differ in mtDNA haplotype and abdominal color; (2) lineage A of brood XXIII is indistinguishable in abdominal color, mitochondrial haplotype, and geographic distribution from the northern lineage of 13-year brood XIX discovered by Martin and Simon (1988; 1990b); (3) northern brood XXIII lineage A is indistinguishable in color and mitochondrial haplotype from all 17-year cicadas examined to date; (4) there is a zone of overlap in brood XXIII where both 13-year cicada lineages A and B cooccur; and (5) genetic evidence suggests that assortative mating has taken place in brood XXIII in the zone of overlap. These findings form the basis of the new evolutionary scenario discussed below.

Complementary to our genetic work is the recent discovery of similar assortative mating in a lineage A and B overlap zone in 13-year brood XIX in the same geographic area as the overlap zone we discovered in 13-year brood XXIII. This finding is supported by data on male song, female response, and abdominal color collected by Marshall and Cooley (2000) 13 years after the initial discovery of brood XIX lineage A

←

between mitochondrial lineages A and B are indicated. Note that in southern Illinois there is a broad zone where lineages A and B overlap, whereas in northern Arkansas the zone is much narrower. (A) 13-year brood XIX; (B) the complete geographic distribution of all 12 17-year cicada broods combined; (C) 13-year brood XXIII.

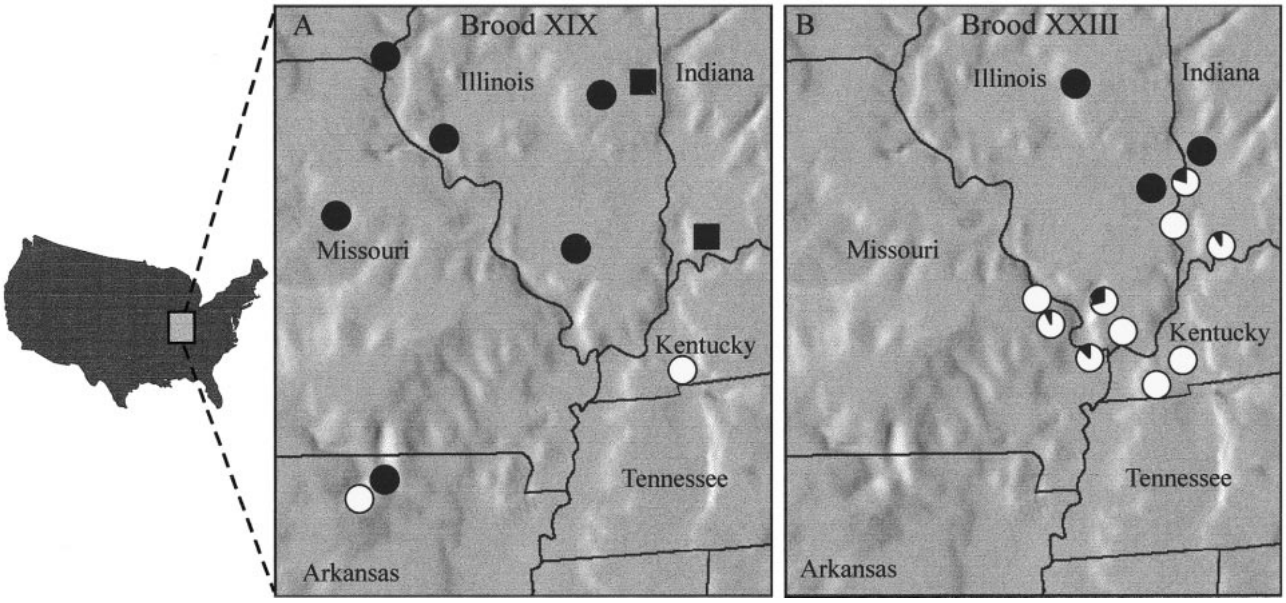

FIG. 2. Zone of contact between the two periodical cicada mitochondrial lineages in two 13-year broods. Pie diagrams show percent of lineage A (dark gray) and lineage B (light gray) mitochondrial haplotypes. (A) Broods X (17-year, squares) and XIX (13-year, circles) redrawn from Martin and Simon (1988), but only showing two of the 10 17-year populations analyzed (five populations from brood X plus one sample each from broods I, IV, V, VII, and IX); (B) brood XXIII (13-year), this study.

TABLE 1. Mitochondrial haplotypes for 198 individuals and mean abdominal color scores for 230 individuals from 15 populations of 13-year periodical cicada brood XXIII with color scores matched to mitochondrial haplotypes in the mixed-lineage populations (note that in the pure-lineage populations the number of individuals color-scored is not equal to the number of individuals haplotyped).

	Mitochondrial haplotype			Color score		
County, state[1]	*N*[2]	Percent (No.) lineage A	Percent (No.) lineage B	*N*[3]	Mean (× 20)[4]	SD
Pure lineage A populations:						
Dewitt Co., IL	29	100 (29)	0 (0)	20	2.06 (41)	0.53
Sullivan Co., IN	24	100 (24)	0 (0)	20	2.44 (49)	1.09
Lawrence Co., IL	12	100 (12)	0 (0)	12	2.48 (50)	0.53
Subtotals	65			52		
Mixed lineage A and B populations						
Jackson Co., IL	14	43 (6)		6	1.67 (33)	1.150
			57 (8)	8	3.91 (78)	0.035
Alexander Co., IL	8	12 (1)		1	2.50 (50)	—
			88 (7)	7	3.28 (66)	0.543
Knox Co., IN	10	30 (3)		3	2.60 (52)	0.794
			70 (7)	7	3.67 (73)	0.522
Cape Girardeau Co., MO	20	4 (1)		1	2.00 (40)	—
			96 (19)	19	3.48 (70)	0.523
Vanderburg Co., IN	10	7 (1)		1	2.00 (40)	—
			93 (9)	9	3.27 (65)	0.638
Subtotals	62			62		
Pure lineage B populations						
Posey Co., IN	22	0 (0)	100 (22)	20	3.38 (68)	0.52
Johnson Co., IL	19	0 (0)	100 (19)	20	3.69 (74)	0.36
Perry Co., MO	8	0 (0)	100 (8)	12	3.11 (62)	0.82
Calloway Co., KY	10	0 (0)	100 (10)	20	3.23 (65)	0.61
Lyon Co., KY	12	0 (0)	100 (12)	20	3.36 (67)	0.56
Pike Co., MS	—	—	—	12	3.66 (73)	0.43
Grenada Co., MS	—	—	—	12	3.68 (74)	0.46
Subtotals	71			116		
Total	198			230		

[1] Exact localities are available from http://www.eeb.uconn.edu/cicadadb.html or the first author.
[2] Number of individuals sampled for mtDNA.
[3] Number of individuals sampled for abdomen color.
[4] Color score multiplied by 20; comparable to values from Martin and Simon (1988).

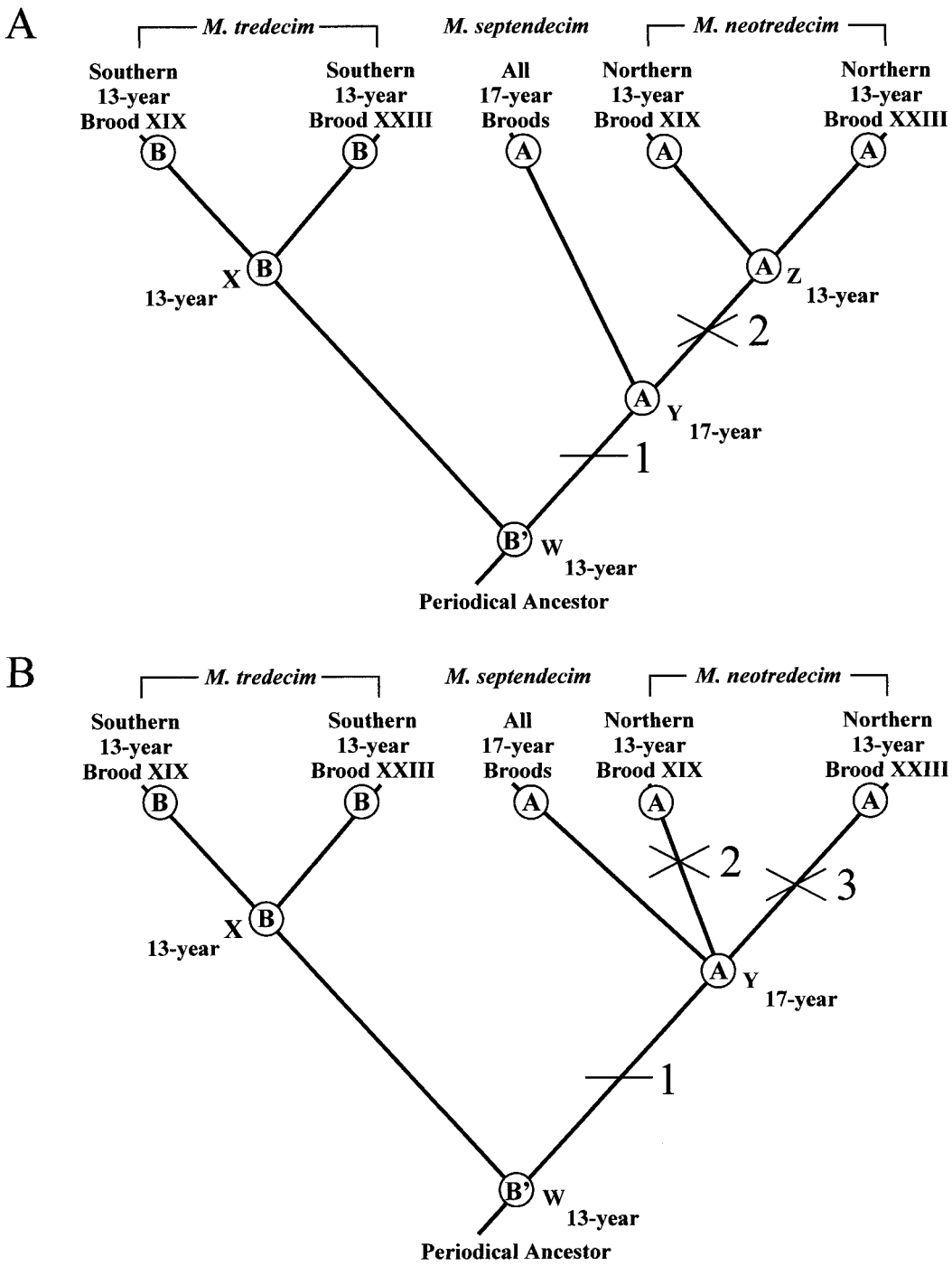

FIG. 3. Hypothesis of the formation of 13- and 17-year decim lineages. *Magicicada tredecim* evolved first (node W), followed by *M. septendecim* (node Y) and *M. neotredecim.* Numbered lines on tree branches mark the addition of four years to the life cycle. Numbered Xs marked on tree branches indicate the deletion of four years from the life cycle. The circled letters A and B at the tips and nodes indicate lineage type. (A) The evolution of *M. neotredecim* involving one evolutionary event (2); (B) the evolution of *M. neotredecim* involving two separate evolutionary events (2 and 3). See text for a detailed explanation.

cicadas by Martin and Simon (1988). Marshall and Cooley named the northern 13-year cicada lineage A cicadas as a new species, *M. neotredecim.* Throughout our discussion, we refer to the northern 13-year lineage A cicadas from both broods XIX and XXIII as *M. neotredecim* and we integrate the results of the two studies.

We propose two possible scenarios for the evolution of the decim species (Fig. 3). In both schemes, the ancestor is a periodical cicada with a 13-year life cycle (node W, lineage B′). This ancestor gave rise to a 13-year proto-brood XIX + XXIII (node X; lineage B), that later split into the current 13-year broods, XIX and XXIII. The ancestor at node W also

gave rise via a four-year life-cycle extension (event 1) to *M. septendecim* (node Y; lineage A). Between nodes W and X and W and Y, some amount of evolution took place at the two marker loci, so that node X evolved lineage B and node Y evolved lineage A. In scenario one (Fig. 3A), *M. neotredecim* evolved from *M. septendecim* via a single four-year life-cycle reversion (event 2), which formed a 13-year proto-brood XIX + XXIII (node Z; lineage A) joining and gaining protection from the existing 13-year proto-brood XIX + XXIII to the south. This combined lineage A + B proto-brood later split into the current broods XIX and XXIII with *M. neotredecim* already in place. In this scheme, nodes X and Z are of the same age. In scenario 2 (Fig. 3B), *M. septendecim* (node Y) contributed *M. neotredecim* at two different times to the preexisting *M. tredecim* broods XIX and XXIII. This involved two independent 17- to 13-year life-cycle reversions (events 2 and 3). In scenario 2, the order of origination of *M. neotredecim* of broods XIX and XXIII from *M. septendecim* is uncertain and node X predates events 2 and 3.

We suggest that in the evolution of periodical cicadas, the 13-year life cycle evolved before the 17-year life cycle because all other cicadas whose life cycles are known develop in fewer than 13 years (Karban 1986). The hypothesis that existing *M. tredecim* evolved before the current *M. septendecim* is supported by the fact that among-population variation is an order of magnitude larger in *M. tredecim* than in *M. septendecim* (Martin and Simon 1990b). Based on a very rough mtDNA clock calibration for insects (Brower 1994), existing *M. septendecim* split from the current *M. tredecim* approximately 1 million years ago.

The event that led to the formation of *M. septendecim* from *M. tredecim* in the first place was likely related to cooler paleotemperatures (Heliovaara et al. 1994; Yoshimura 1997; Cox and Carleton 1998). Temperature variation is known to alter insect diapause duration (e.g., Powell 1989; Heliovaara et al. 1994). The four-year life-cycle-length difference is hypothesized to have been added in one jump because no periodical cicadas with intermediate life cycles have ever been found and gradual lengthening would lead to increased mortality from predation a year or two following a big emergence (Williams and Simon 1995; Yoshimura 1997; but see Cox and Carleton 1998). Acceleration could be accomplished by the deletion of the well-documented four-year dormancy in the second instar of 17-year cicadas (White and Lloyd 1975). A four-year jump would also decrease competition with established underground nymphs of the source brood (Simon et al. 1981). We suggest that the initial formation of *M. septendecim* occurred allopatrically, or at least parapatrically, when the life cycles of northern populations were lengthened due to the colder climates of the Pleistocene.

The current broods of 17-year cicadas appear to be post-Pleistocene based on their geographic distribution and lack of genetic differentiation from each other (Martin and Simon 1990b). We suggest that following the four-year life-cycle switch that produced them, *M. septendecim* expanded quickly, moving north with advancing deciduous-tree fronts tracking the retreat of the glaciers and the northern spruce-fir forest. This explains the lower genetic variation seen north of the glacial/spruce-fir boundary (Martin and Simon 1990b). This pattern of genetic ''northern purity'' versus ''southern richness'' is seen in dozens of Palearctic and Nearctic species that have invaded formerly glaciated terrain (Hewitt 1996).

In both evolutionary scenarios (Fig. 3), we hypothesize that lineage A arose in *M. septendecim* first and that *M. neotredecim* was a secondary derivative, rather than vice versa. Evidence for the later derivation of *M. neotredecim* is its location entirely above the last glacial boundary of habitable deciduous forest in a small gap in the spatial distribution of all 17-year cicadas (Fig. 1). Furthermore, allochronic isolation of 17-year *M. septendecim* from 13-year *M. tredecim* cicadas could explain the differentiation of lineage A from lineage B. In contrast, to hypothesize that lineage A evolved in *M. neotredecim,* an unknown barrier between 13-year cicadas of lineages A and B would need to be proposed. In addition, it seems unlikely that *M. neotredecim* would have later produced *M. septendecim* as it moved out from this central location to colonize eastern, northern, western, and finally southern locations across no recognizable geographic or geological boundary.

The most likely progenitor of *M. neotredecim* is brood X. It is the only 17-year brood that is parapatric to both broods XIX and XXIII and it was previously recorded in the range of *M. neotredecim* (Fig. 1B, gray dots; Simon 1988). The life-cycle reversion(s) that formed *M. neotredecim* must have occurred relatively recently because we have found no genetic difference in mitochondrial DNA and abdominal color between *M. neotredecim* and *M. septendecim* (see also Martin and Simon 1988, 1990b).

We suggest that the mechanism for the persistence of *M. neotredecim* involves the peculiar predator satiation strategy of periodical cicadas (Lloyd and Dybas 1966; Karban 1982; Williams et al. 1993). *Magicicada neotredecim* occur only in broods that include *M. tredecim*; in both broods in which the two species have been found, they are contiguous. We propose that only when four-year accelerations from *M. septendecim* occurred adjacent to preexisting *M. tredecim* would the accelerating individuals survive predation. This mechanism is not new. Alexander and Moore (1962) speculated that the co-occurring decim, decula, and cassini species each gain protection from predation by the presence of the others. Lloyd and Dybas (1966, p. 495) extended this idea and suggested that ''a species present in one brood but lacking in another could become 'inducted' into the second brood'' in areas where the two broods were adjacent or sympatric. A similar explanation was used by Marshall and Cooley (2000), who coined the helpful term ''nurse-brood facilitation.'' Marshall and Cooley also report that reproductive character displacement in male song and female preference occurred at the zone of contact. They suggest that this was due to selection against rare hybridization events or simply time wasted in ineffectual courtship activities (Marshall and Cooley 2000). Thus, it appears that when *M. neotredecim* joined *M. tredecim,* they already differed sufficiently to be reproductively isolated.

One of the most significant findings of our work is that both broods XIX and XXIII contain lineages A and B. We have presented two alternative scenarios under which this may have happened. In both, we presume that the transfer of lineage A into 13-year cicadas occurs by the induction or nurse-brood facilitation method described above. In the first

scenario, the induction occurs between 17-year brood X and some 13-year proto-brood XIX + XXIII during years of coemergence. Later, this proto-brood splits into the present broods XIX and XXIII. In the second scenario, the induction of lineage A into broods XIX and XXIII occurred independently during their respective years of coemergence with brood X. We have no genetic or behavioral data to distinguish between the two hypotheses. Although the single life-cycle reversion suggested by the first scenario is more parsimonious, we can think of no biological reason to suspect that induction would not occur every time a 13- and 17-year brood coemerged.

In both our schemes, the life-cycle-switching cicadas would have survived progressively farther north each time the accelerating 17-year cicadas coemerged with a 13-year brood or proto-brood to the south (i.e., once every 221 [13 × 17] years). These can be thought of as successive instantaneous, allochronic events gradually enlarging the range of *M. neotredecim.* In the life-cycle-switching zone, any remaining 17-year cicadas have since gone extinct either through competition (Lloyd et al. 1983), predation, or weather-related phenomena or through some combination of these factors. As noted earlier, the fact that 13-year lineage B and 17-year lineage A cicadas are indistinguishable at the two marker loci and the fact that *M. neotredecim*'s range is carved out of the center of the range of their 17-year siblings and lies in terrain that was uninhabitable during glacial times, argues for a recent origin—since the last glacial maximum.

Successful four-year switches from 13- to 17-year life cycles and back must be relatively rare because, if they were common, there would be no genetic differences between the decim lineages. Genetic exchange between life-cycle types every 221 years must be rare today because few overlap areas exist. For example, *M. septendecim* and *M. neotredecim* overlap only in a 10 × 30 km region in southeastern Iowa (Lloyd et al. 1983) and *M. cassini* and *M. tredecassini* overlap only in a similarly small region of southeastern Oklahoma. We do not know how much overlap occurred in the past, but all 13- and 17-year cicada species now seem to be on separate evolutionary trajectories.

Although our data strongly support the importance of four-year accelerations in periodical cicada evolution, they provide no new clues to understanding the actual mechanism of operation. Four-year accelerations have been hypothesized to be triggered by either hybridization (Lloyd et al. 1983) or underground nymphal competition (Lloyd and White 1976), but may in fact be caused by some other mechanism. The fact that no evidence of widespread hybridization has been found in northern, pure lineage A populations of *M. neotredecim* of broods XIX and XXIII argues against the hybridization hypothesis, as Martin and Simon (1988) pointed out. Genetic control of the life cycle is unknown, but has been hypothesized to be a single-locus/two-allele system with either the 17-year allele (Lloyd et al. 1983) or the 13-year allele (Cox and Carlton 1991) dominant. Below, we suggest that four-year accelerations occur among the offspring of all female *M. septendecim.*

One question about periodical cicada four-year accelerations that has plagued evolutionary biologists and is central to this paper is, ''why hasn't natural selection eliminated mistakes in timing?'' In other words, why do four-year acceleration events occur? Life-cycle switching events are often assumed to be lethal due to insufficient coemerging cicadas to provide protection from predation (Williams and Simon 1995). Thus, selection should act to favor individuals whose offspring all emerge close together in time. This is contrary to the general situation in insects where bet-hedging or risk-spreading (Hopper 1999) can have a strong selective advantage because the offspring of any given female emerge over an extended time period, as insurance against poor conditions at any one time period. Williams and Simon (1995) suggested that periodical cicadas had to give up the selective advantage of bet-hedging to become periodical. We suggest that, although for the most part this is true, periodical cicadas retained a vestige of bet-hedging in the form of four-year accelerations. We suggest that four-year accelerations occur regularly in periodical cicada populations in a small percentage of the offspring of most individual females. Although many accelerating individuals die, enough survive by joining existing broods to provide a selective advantage to this life-cycle plasticity. Because each female decim can lay 400 to 600 eggs and because mortality is high (Karban 1986), losing a small percentage of offspring to acceleration may provide more benefit than risk. Perhaps some fraction of these accelerating offspring are always permanent 13-year cicadas, whereas others are temporary accelerators and revert to a 17-year life cycle after one generation, fitting both of the four-year acceleration patterns that have been observed in nature (Lloyd and White 1976; Simon et al. 1981; Simon and Lloyd 1982; Kritsky 1988, 1992; Marshall and Cooley 2000). Unfortunately, bet-hedging is even more difficult to test in periodical cicadas than it is in shorter-lived insects.

Should 13- and 17-Year Cicadas Be Called Different Species?

Biologists since Charles Darwin (Darwin 1903) have wrestled with the application of species concepts to periodical cicadas. Darwin felt that 13- and 17-year cicadas should not be designated separate species unless some difference other than the life cycle could be found. Armed with a greater knowledge of the biology of *Magicicada,* Alexander and Moore (1962) felt that life-cycle differences were sufficient for species designation. Working within the framework of the biological species concept, they suggested that, in general, species should be designated as soon as they are distinguishable if it is likely that the distinguishable populations will remain extant and isolated long enough for reproductive isolation to develop. They believed that there was sufficient evidence for a severe restriction in gene exchange between life cycles because: (1) specimens of 13- versus 17-year decim could usually be distinguished by abdomen color and this difference is ''no less pronounced where the two species overlap''; (2) no intermediate life cycles are found in areas where 13- and 17-year cicadas overlap; and (3) the zone of geographic overlap between 13- and 17-year cicadas is very narrow.

Life-cycle switching has resulted in a natural experiment where 13-year cicadas (*M. neotredecim*) recently derived from 17-year cicadas (*M. septendecim*) now co-occur with a

preexisting 13-year lineage (*M. tredecim*). Martin and Simon (1988, 1990a; Williams and Simon 1995) suggested that such shifts in life-cycle timing would reunite the gene pools of *M. tredecim* and *M. septendecim,* invalidating the species status of this 13-/17-year pair. This conclusion was based on a lack of behavioral barriers to reproduction in experimental matings (Lloyd and Dybas 1966). These experiments were conducted in crowded conditions. The behavioral experiments of Marshall and Cooley (2000) combined with extensive field observations now demonstrate that important differences do exist in male song pitch and female response between *M. neotredecim* and *M. tredecim.*

We agree with Marshall and Cooley's (2000) designation of *M. neotredecim* as a new species. Their rationale for species status of *M. neotredecim* compared to *M. tredecim* is the difference in both male and female mating signals and the clear reproductive character displacement. Their rationale for species status of *M. neotredecim* compared to the behaviorally and genetically identical *M. septendecim* is limited geographic and temporal overlap and the unlikelihood of reversion of *M. neotredecim* to a 17-year life cycle synchronized with any extant 17-year broods before reproductive isolation is achieved. Furthermore, the name *M. neotredecim* is extremely useful for discussing the evolution of the group and an excellent choice because it reflects the recent evolution and secondary nature of this lineage.

Finally, Marshall and Cooley's (2000) behavioral observations significantly strengthen our hypothesis of assortative mating. Our supposition was based on a correlation of abdominal color and mitochondrial genotype. We did not obtain mating pairs and assess their genotypes. With the addition of their data, it now seems clear that assortative mating is taking place. This does not mean, however, that no hybridization is occurring. Evidence from the few odd abdomen/haplotype combinations we found suggest that either there has been some hybridization or that abdominal coloration mutants are more common than we suspect. Gene exchange does not necessarily invalidate species status. Even under the biological species concept, many recognized species exchange genes (Arnold 1997). We are currently in the process of quantifying gene flow among *M. tredecim, M. neotredecim,* and *M. septendecim* lineages.

The *M. tredecim/neotredecim* contact zone could be similar to that observed by Jiggins et al. (1997) for *Heliconius erato* and *H. himera.* In these two species, parental genotypes at a number of unlinked gene loci remain in tact despite some hybridization (5–10%) and no apparent hybrid inviability. In their case, as in ours, they suggest that strong mate preferences are responsible for the rarity of detectable hybrid phenotypes.

Species concepts (e.g., Cracraft 1989; Templeton 1989; Baum and Shaw 1995; Mallet 1995) have been a magnet for controversy in evolutionary biology because species designations depend heavily on one's view of how species form and how they should be recognized and named. This controversy is extremely useful for clarifying our understanding of how evolution works. We argue that equally important to the designation of species names and to the decision as to when speciation occurs is the explanation of the evolutionary steps leading to the current distribution of genetic variation within and among groups of populations. Part of a good evolutionary explanation is the characterization of mating behavior and genetic variation at marker loci within and among populations. This work is ongoing in our laboratory.

Conclusion

Speciation mechanisms are a major focus of evolutionary research (Coyne 1992) because speciation is the process by which global biodiversity is generated. One general class of speciation mechanisms, instantaneous speciation, is of interest because it can lead to differentiation in sympatric populations without the difficult problem of counteracting gene flow, which plagues noninstantaneous sympatric speciation models. Instantaneous speciation has been proposed to occur by three different processes: chromosome duplication (polyploidy; Stebbins 1950), parasitic infection (Laven 1959), or shifts in reproductive timing (allochrony; Mayr 1947). Although each of these mechanisms was proposed more than 50 years ago and discussed widely, only instantaneous speciation by polyploidy (in plants) is genetically well documented (Lewis 1979). Instantaneous speciation by parasitic infection is still uncertain, but recent data suggest it has occurred in insects infected by parasitic bacteria (Bordenstein and Werren 1998). Instantaneous speciation by allochrony was suggested for periodical cicadas (e.g., White 1978), but remained speculative until now. The periodical cicada case is also significant because few good examples of allochronic speciation exist (White 1978), fewer have been supported by genetic data (Wood and Guttman 1982; Feder et al. 1993; Harrison and Bogdanowicz 1995), and no others are thought to have been instantaneous.

Periodical cicadas are also unique in that all other putative examples of allochronic shifts (e.g., Alexander and Bigelow 1960; Wood and Guttman 1982; Feder et al. 1993) are proposed to have acted to separate rather than reunite lineages. We hypothesize that an initial allochronic event separated lineage A and B periodical cicadas and a second allochronic event placed them in secondary contact. Neither four-year accelerations nor allochronic speciation are new concepts in periodical cicada evolution, however, our genetic data provide empirical support for creative ideas that were previously based only on circumstantial evidence and speculation (Marlatt 1907; Alexander and Moore 1962; Lloyd and Dybas 1966). We also provide a new hypothesis for four-year accelerations in the form of bet-hedging and provide genetic evidence for assortative mating.

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