the observed divergence (at least 5.2%) may have accumulated over a span of about 5×10^6 yr. This contrasts with most clonal species of hybrid origin, in which divergences from the maternal parental species are usually less than 1%. The deduced maximum age of other clonal vertebrate lineages is thus considerably less than that of clonal *Ambystoma*.

The long-term persistence of clonal species of *Ambystoma* provides independent evidence that the germ-line modifications that allow their persistence from generation to generation are also effective in the long term. Given the apparent great age of these clonal lineages, the very low diversity (in both number of haplotypes and number of nucleotide differences) is surprising. The uniformity of mtDNA suggests that the mtDNA of all extant clonal lineages is derived from a single sexual female. If two or more sexual females with identical mtDNAs had been involved in the original hybridizations some 5×10^6 yr ago, the mtDNAs in their clonal descendants would subsequently have followed separate evolutionary trajectories and therefore would have diverged much more than 0.1%.

The very low clonal diversity could be a consequence of either selection or of stochastic processes resulting in severe real or effective restrictions in the number of reproducing clonal individuals. Selection (compare with ref. 28) seems an unlikely explanation for three reasons: (1) because both nuclear and mitochondrial genomes in a clonal organism would face the same selection process, there should be no nuclear genetic variability; (2) selection should not act on silent substitutions, so restriction site changes involving them should still occur; (3) selection requires both migration and competitive replacement, population by population, of all existing haplotypes by the favoured haplotype, a slow process. Alternatively, severe geographic population restriction requires only subsequent emigration and expansion. Population restriction is plausible given the biogeography of the *A. jeffersonianum* complex: *A. platineum* and *A. tremblayi* occur almost exclusively in areas covered by the Wisconsin glaciation; these taxa may have survived only in small refugia during each of the Pleistocene glaciations and repopulated glaciated areas during each interglacial period.

The diversity in nuclear genotypes of clonal lineages may reflect infrequent replacement of nuclear genomes in clonal species by genomes from males of the sexual host species. That *A. platineum* and *A. tremblayi* both have both clonal haplotypes suggests that such genome replacement through male hosts may have occurred, although replacement has not been demonstrated in natural populations of *Ambystoma*, and must be infrequent[29]. Even rare replacement, however, may be important in maintaining the nuclear variability present in clonal *Ambystoma* and thus in increasing long-term survival of clonal salamander lineages. □

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Ancestry of unisexual salamanders

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IN eastern North America there are populations of all-female salamanders that incorporate the nuclear genomes of two or three of four sympatric bisexual species. The hybrids can be diploid, triploid, tetraploid or pentaploid, and 18 different combinations have been reported. All hybrids require sperm from a sympatric male of one of the bisexual species to reproduce, but the sperm may or may not be incorporated in the egg. Some of the hybrids are believed to represent separate, clonal species, but little is known of the origin of this hybrid complex. Vertebrate mitochondrial DNA is inherited maternally, allowing identification of the female parent that gave rise to hybrid lineages. A portion of the cytochrome *b* gene was sequenced from diploid and triploid hybrids that represent combinations of all four species. Nearly all hybrids had a similar mitochondrial genome sequence, independent of nuclear genome composition and ploidy, and the sequence was distinct from that of any of the four bisexual species. The hybrids maintain a mitochondrial lineage that has evolved independently of their nuclear genome and represent the most ancient known unisexual vertebrate lineage.

To determine the maternal ancestry of the unisexual salamander (genus *Ambystoma*) hybrids, we sequenced part of the mitochondrial (mt) cytochrome *b* gene from 46 salamanders, including diploid and triploid hybrid combinations of all four bisexual species (*A. jeffersonianum*, *A. laterale*, *A. texanum*, *A. tigrinum*; ref. 1). The cytochrome *b* gene evolves sufficiently rapidly to allow determination of the relationships among closely related populations and species of vertebrates[2]. We extracted and purified DNA from salamanders with known chromosome numbers and allozyme genotypes[3–7]. To compare the bisexual species with these hybrids, we chose samples of *laterale*, *jeffersonianum*, *texanum* and *tigrinum* from widely separated populations, to examine the range of sequence differentiation within these species. The hybrid samples included diploid *laterale* × *texanum*, where the two species occur sympatrically, and diploid *laterale* × *jeffersonianum*, where the two species occur parapatrically. The triploid combinations *laterale* × 2-*jeffersonianum* (LJJ), 2-*laterale* × *jeffersonianum* and *laterale* × *texanum* × *tigrinum* (LTTi), believed to represent separate clonal species[8–10], were also included. The triploid hybrids *laterale* × 2-*texanum* 2-*laterale* × *texanum*, and LTTi were chosen to include the same hybrid combinations that had been used in restriction fragment length polymorphism (RFLP) analyses[11,12], as well as LJJ which had not been previously examined. Some of the individual triploid hybrids chosen demonstrated genetic exchange of diagnostic electromorphs[4]. An additional species, *A. mexicanum*, was included for comparison, and the plethodontid species *Plethodon yonahlossee* was used as an outgroup. A region of 307 base pairs of the cytochrome *b* gene in each salamander was amplified and sequenced (Fig. 1).

Of the 20 hybrids, 18 form a separate monophyletic lineage

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                 0000000000000000000000000000000000011111111111111111111111111111111111111122222222222222222222222222222222222222222223333
                 000001111112222233444556777888889999000011122233334444556666777788899999000001111222223344445555566666677778899990000
                 1347901234902568570690610691245784780369258147035792458570369024817901369124891457025690201470236902358923470602581247
 1 Plethodon     TTTCCCTTGCAGCCCTCATCATTACATTTACTCTATAAATCCACACCAGTATGATACCACTCATCTTATAACCGAGAATTCTACTTACTCCATCGAACCTTTAAACTAACAAATCTCA
 2 L-Nort-Ont    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..T..AG.T.NT.TACATTGTA..TG..CTCT.
 3 L-PrinEdId    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..TC.AG.T..T.TACATTGTA..TG..CTCT.
 4 L-PeId-Ont    C..T.T..AT.CA.TA..CA...TT.CCACACA.G.TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..T..AG.T..T.TANATTGTA..TG..CTCT.
 5 L-Sou1-Ont    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..T..AG.T..T.TACATTGTA..TG..CTCT.
 6 L-Sou2-Ont    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..T..AG.T..T.TACATTGTA..TG..CTCT.
 7 L-Moos-Ont    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..T..AG.T..T.TACATTGTA..TG..CTCT.
 8 L-Shelb-VT    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..TC.AG.T..T.TACATTGTA..TG..CTCT.
 9 L-NewLN-CT    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATTCT..TCCT...C...ACT.C.A..TC.AG.T..T.TACATTGTA..TG..CTCT.
10 L-LongI-NY    C..T.T..AT.CA.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..TC.AG.T..T.TACATTGTA..TG..CTCT.
11 L-Illinois    C..T.T..AT.CT.TA..CA...TT.CCACACA...TGT.TTGT...C.CTA...GATGTATT.TC.TCCT...C...ACT.C.A..T..AG.T..T.TACATTGTA..TG..CTCT.
12 J-Sou1-Ont    C.CT.TC.A.GCGTTA..CA.CCTTTCCACACA...C..C.......C.CTA...GA..TATT....T.CTT..C...ACT...AC.T.GAG....TTTACATTGTAG.TG...TCTG
13 J-Sou2-Ont    C.CT.TC.A.GCGTTA..CA.CCTTTCCACACA...C..C.......C.CTA...GA..TATT....T.CTT..C...ACT...AC.T.GAG....TTTACATTGTAG.TG...TCTG
14 J-Sou3-Ont    C.CT.TC.A.GCGTTA..CA.CCTTTCCACACA...C..C.......C.CTA...GA..TATT....T.CTT..C...ACT...AC.T.GAG....TTTACATTGTAG.TG...TCTG
15 J-Connecti    C.CT.TC.A..CGTTA..CA.CCTTTCCACACA...C..C.......C.CTAC..GA..TATT....T.CTT.CC...ACT.G.AC.T..AG.TC.TTTACATTGTAG.TG...TCTG
16 J-Dutch-NY    C.CT.TC.A..CGTTA..CA.CCTTTCCACACA...C..C.......C.CTA...GA..TATT....T.CTT..C...ACT.G.AC.T..AG.T..TTTACATTGTAG.TG...TCTG
17 J-Tompk-NY    C.CT.TC.A.GCGTTA..CA.CCTTTCCACACA...C..C.......C.CTA...GA..TATT....T.CTT..C...ACT...AC.T.GAG....TTTACATTGTAG.TG...TCTG
18 T-PeI1-Ont    ......C.A..TA.TA.GCA...TT.CCACACA...T......T.TTTCCTA..CGA.CTATC.T.CT.CTTT.C...GCTCC.A..T..A.CA....TACATTGTA..TG...GCT.
19 T-PeI2-Ont    CCCT..C.AT.TA.TAGGCA...TT.CCACACA...T......TGTTTCCTA..CGA.CTATC.T.CT.CTTT.C...ACTCC.A..T..A.C.....TAGATTGTA..TG...GCT.
20 T-Missour1    ........A..CG.TA.GCAG..TT.CCACACA...T......T.TTT.CTA..CGATCTATC.T.CT.CTTT.CA..GCTCC.A..T..A.CT....TACATTGTA..TG...GCT.
21 T-Missour2    CCCT..C.AT.TA.TAGGCA...TT.CCACACA...T......T.TTTCCTA..CGATCTATC.T.CT.CTTT.T...ACTCC.A..T..A.C.....TACATTGTA..TG...GCT.
22 mexicanum     ...A.TC.AT.TA.TA..CA...TT..CACACAC..C.CCT..T.TTT.CTA...GA.CTATC.TCCT.CTTT.T...A.TCC.A.GTC.A.CT...TTA.ATCGT...TGT..TCT.
23 Ti-KeI1-OH    ...A.TC.AT.TA.TA..CAG..T...CACACA.....T..T.T.T.T.CTA.T.GATCTATC.T..T.CTTT.C...A.TCC.AC.T..A.CT...TTACATCGT...TG.G.TCT.
24 Ti-KeI2-OH    ...A.TC.AT.TA.TA..CAG..T...CACACA.....T..T.T.T.T.CTA.T.GATCTATC.T..T.CTTT.C...A.TCC.AC.T..A.CT...TTACATCGT...TG.G.TCT.
25 Ti-Illino1    ...A.TC.AT.TA.TA..CAG..TT..CACACA.....T..T.T.TTT.CTA.T.GATCTATC.T..T.CTTT.C...A.TCC.AC.T..A.CT...TTACATCGT...TG.G.TCT.
26 Ti-Illino2    ...A.TC.AT.TA.TA..CAG..TT..CACACA.....T..T.T.TTT.CTA.T.GATCTATC.T..T.CTTT.C...A.TCC.AC.T..A.CT...TTACATCGT...TG.G.TCT.
27 LJ-Sout-On    C.......A..CA.TA..CAG..TT.CCACACA...T....T.T...C.CTA...GA...ATC....T.CTT..T.TTACTCC.A..T..A..T.G...ACACCGTA.TTG...TCT.
28 LJ-Suff-CT    C....T..A..CA.TA..CAG..CT.CCACACAC..T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T.TTACTCC.A..TC.A....T...ACACTGTA.TTGT..TCT.
29 LJ-Dut1-NY    ........A..TA.TA..CAG..CT.CCACACA...T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T.TTACT.C.A..TC.A.C..T...A.ACTGTA.CTGT..TCT.
30 LJ-Dut2-NY    C......GA..CA.TA..CAG..CT.CCACACAC..T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T.TTACT.C.A..TC.A.C..T...ACACCGTA.CTGT..TCT.
31 LT-PeI1-On    ...A.TC.A..CA.TA..CAG..TT.CCACACA...T....T.T.T.C.CTA...GA.C.ATC.T..T.CTTT.T.TTACTCC.A..T..A.CT.....ACATTGTA.TTG...TCT.
32 LT-PeI2-On    ......C.A..CA.TA..CAG..TT.CCACACA...T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T.TTACTCC.A..T..A.C.....TAGATTGTA.TTG...TCT.
33 LT-PeI3-On    ......A.A..CA.TA..CAG..TT.CCACACA...T....T.T.T.C.CTA...GATC.ATC.T..T.CTTT.T.TTGCTCC.A..T..A.CA....TACACTGTA.TTG...TCT.
34 LJJ-S1-Ont    ....G.C.A..CA.TA..CAG..CT.CCACACA...T....T.T...C.CTA...GA...ATC....T.CTTT.T.TTACTCC.A..T..A.C..T...ACACTGTA.CTGT..TCT.
35 LJJ-S2-Ont    C.....C.A..CA.TA..CAG..CT.CCACACA...T....T.T...C.CCA...GA.C.ATC....T.CTTT.T.TTACTCC.A..T..A..T.TC..ACACTGTA.CTGT..TCT.
36 LJJ-S3-Ont    C.....C.A..CA.TA..CAG..CT.CCACACAC..T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.C.TTACTCC.A..TC.A.C..T...ACACTGTA.CTGT..TCT.
37 LJJ-Har-CT    C.....C.A..CA.TA..CAG..CT.CCACACAC..T....T.T...C.CCA...GA.C.ATC....T.CTTT.T.TTACTCC.A..T..A..T.G...ACACTGTA.CTGT..TCT.
38 LJJ-Col-NY    C....GC.A..CA.TA..CAG..CT.CCACACA...T....T.T...C.CCA...GA...ATC.T..T.CTTT.T..TACTCC.A..T..A.......NACATCGTA.TTG...TCT.
39 LLJ-So-Ont    ........A..TA.TA..CAG..CT.CCACACAC..T....T.T...C.CCA...GA.C.ATC....T.CTTT.T.TTACTCC.A..TC.A..T.G...ACACTGTA..TGT..TCT.
40 LLJ--Maine    ........A..TA.TA..CAG..CT.CCACACA...T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T.TTACTCC.A..TC.A.C..T...A.ACTGTA.CTGT..TCT.
41 LLJ-GId-VT    C.....C.A..CA.TA..CAG..CT.CCACACAC..T....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T..TACTCC.A..TC.A.CT.T...A.ACTGTA.CTGT..TCT.
42 LLJ-She-VT    ........A..TA.TA..CAG..CT.CCACACA...T....T.T...C.CCA...GA.C.ATC.T..T.CTTT.T..TACT.C.A..TC.A.CT.T...A.ACTGTA..TGT..TCT.
43 LLT-P1-Ont    ........A..TA.TA..CAG..CT.CCACACA..CT....T.T.T.C.CCA...GA.C.ATC.T..T.CTTT.T.TTACTCC.A..TC.A.CA.T...ACACTGTA.CTGT..TCT.
44 LTT-P2-Ont    ........A.CNA.TAN.CA...CT.CCACACA...T....T.T.T.C.CTA...GA.CTATC.T.AT.CTTT.T..TACTCCAA..TCAA.C.....TAGATTGTA.TTGN..TCT.
45 LTT-KI-Ont    ........A.CTA.TA..CA...CT.CCACACA...T....T.TGT.C.CTA...GA.CTATC.T..T.CTTT.C...ACTCC.A..T..A.CT....TACATCGT...TG.G.TCT.
46 LTTi-KI-OH    ...A.TC.A..TA.TA..CAG..CT..CACACA...GGT..T.TGT.T.CTA...GA.CTATC.T..T.CTTT.C...ACTCC.A..T..A.CT....TAGATCGTA..TG.G.TCT.
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FIG. 1 Mitochondrial DNA sequence variation from 307 base pairs of the cytochrome *b* gene in 46 salamanders, corresponding to sites 14,842–15,148 in the human sequence[13]. Only the 118 variable sites are shown, each identified (top) by the 3 digits that give its sequence location. Dots represent sequence identity with *Plethodon* (outgroup); N denotes ambiguities. Species are denoted Ti, *tigrinum*; T, *texanum*; L, *laterale* and J, *jeffersonianum*. Combinations of two or three abbreviations represent diploid or triploid hybrids, respectively. Sequences have been deposited in the EMBL database, accession number X63557. Specimens were from a wide range of localities (see Fig. 2).
METHODS. DNA was extracted from the liver homogenate samples (−70 °C) used in allozyme analyses. Extraction, amplification (polymerase chain reaction) and sequencing followed standard protocols[19,20] using redesigned primers: 5′-CCAACCCCATCAAACATTTCATCATTATGAAA-3′ and 5′-ACTGTAGCCCCTCAAAAAGATATTTGTCCTCA-3′. Sequence data were read from autoradiograms and aligned using the multisequence editing program ESEE[21].

in the phylogenetic tree independent of ploidy, nuclear genomic composition, or locality (Fig. 2). The maternally derived mt DNA found in the hybrids is not from any of the four contemporary species but seems to derive from a distinct ancestral lineage. None of the 20 hybrids clustered with any of the 4 bisexual species. Analysed as characters, shared-derived nucleotides at six sites are present in all or a majority of the hybrids whereas no shared-derived sites join the hybrid lineage with *texanum* as was previously suggested[11,12]. The four *texanum* individuals from two widely separated localities cluster as a monophyletic group at a high bootstrap *P* value (96%; Fig. 2). As expected, most of the DNA sequence differences are transitions at synonymous sites, although one site defining the major hybrid lineage is a transversion involving an amino-acid replacement (T to A resulting in methionine replacing leucine in the protein at a site corresponding to 15,050 in the human DNA sequence[13]). The two 'outlying' hybrids from Kelley Island may

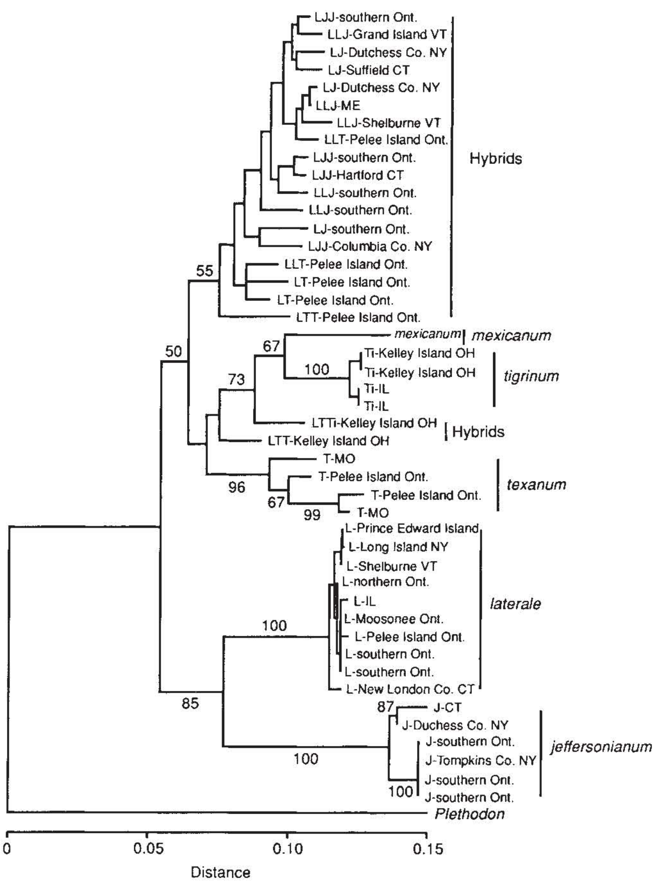

FIG. 2 Evolutionary tree of the unisexual hybrid *Ambystoma* salamander complex based on mtDNA sequence data. The neighbour-joining method[22,23] was used with the Jukes–Cantor distance[24] (scale bar); bootstrap *P*-values[25] are indicated on the tree; abbreviations are as in Fig. 1. Maximum parsimony and UPGMA analyses resulted in the same groups as indicated by vertical bars and in the same relationships, except that in the parsimony tree *texanum* was a sister group to *tigrinum* + *mexicanum* + hybrids, and in the UPGMA tree the two Kelly Island hybrids clustered and formed a sister group to *tigrinum*.
METHODS. Location of specimens: *laterale*, James Bay, Ontario Moosonee, southern Ontario (Ont.) northern Illinois (IL) Pelee Island in Lake Erie, Prince Edward Island, Vermont (VT), Connecticut (CT) and New York (NY); *jeffersonianum*, Ontario, Connecticut and New York; *texanum*, Missouri (MO) and Pelee Island, Ontario; and *tigrinum*, Kelley Island, Ohio (OH) and from northern Illinois. The programs NJTREE (L. Jin), TDRAW (W. Ferguson, University of Texas) and NJBOOT (T. S. Whittam) were used to construct the neighbour-joining tree and perform the bootstrap analysis (2,000 replications). The program UPGMA (J. C. Stephens) was used to construct a tree and to estimate the time of origin (±2 standard errors) of the major hybrid lineage. The program PAUP (D. Swofford) was used to construct a maximum parsimony tree.

provide evidence for additional hybridization events but the data are insufficient to resolve branching order in that part of the tree.

These results are unexpected and differ from the allozyme results used to document the nuclear genomic content of the hybrids. If the female parent of a hybrid had been any one of the four species examined, the hybrid would be expected to cluster with that species. Our results confirm the uncoupling of mitochondrial and nuclear genomic evolution in this hybrid complex that was suggested by RFLP analyses of mitochondrial DNA (mtDNA)[11,12]. However, we find the hybrids to be an ancient lineage (or lineages) that is not closely related to *texanum* as was suggested by these RFLP studies (an ancient origin for some unisexual *Ambystoma* has also been suggested by C. Spolsky, personal communication). The allozyme data show there are one or more *laterale* haploid chromosome complements in every hybrid but none of the hybrid mtDNA sequences clusters with *laterale* mtDNA sequences. Consistent with these findings, the hybrid mtDNAs exhibit considerable differentiation, as much or more than that observed within each of the bisexual species (Fig. 2). We attribute these differences from earlier results to the inclusion in this study of all four bisexual species involved in the hybrid complex, a broader geographic sampling of hybrids and bisexuals and greater resolution of genetic variation by the sequence data.

Our results answer two important, lingering questions concerning the evolution of this intriguing salamander complex: (1) there are no hybridizations where both parents are among the four bisexual species because none of the hybrids clusters with any of these species; and (2) individuals of the pure species are probably not reconstituted from the hybrids[7,14], because none appears within the hybrid cluster. This large difference in the evolution of mtDNA and nuclear DNA has not been reported in other unisexual vertebrates[15] or in any other organism. We hypothesize that the mtDNA of the hybrids has been transmitted clonally from a very distant ancestor while the hybrids continue to acquire nuclear material from the four bisexual species.

To estimate the time of origin of the main unisexual hybrid lineage (Fig. 2), we used the calibration for cytochrome *b* sequence divergence (2.5% per million years) applied in other vertebrates[2,16] and obtained a date of 3.9 ± 0.6 million years ago (Pliocene; independent calibration within *Ambystoma* is not possible as the fossil record is poor). The widely separated samples of *laterale* have nearly identical sequences, implying a recent origin (<200,000 years ago) which agrees with molecular data for other northern salamanders that have undergone rapid, postglacial range expansion[17]. The hybrids do not show such high sequence similarity; their sequence divergence suggests that multiple sublineages have existed for several million years. In contrast, the oldest unisexual vertebrate lineage previously reported, a population of Mexican poeciliid fish, is believed to be only 60,000–150,000 years old[18].

Hybrid *Ambystoma* represent only one of a number of unisexual vertebrate complexes[1] and it will be interesting to see if other unisexuals have independently evolving nuclear and mitochondrial genomes. It is instructive to know that the mt DNA need not co-evolve with the nuclear genome and that phylogenies based on mtDNA may be very different from those derived from nuclear genes. □

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Genetically engineered alteration in the chilling sensitivity of plants

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THE chilling sensitivity of plants is closely correlated with the degree of unsaturation of fatty acids in the phosphatidylglycerol of chloroplast membranes[1–5]. Plants with a high proportion of *cis*-unsaturated fatty acids, such as spinach and *Arabidopsis thaliana*, are resistant to chilling, whereas species like squash with only a small proportion are not. The chloroplast enzyme glycerol-3-phosphate acyltransferase seems to be important for determining the level of phosphatidylglycerol fatty acid unsaturation[6–9]. Here we report that the level of fatty acid unsaturation of phosphatidylglycerol and the degree of chilling sensitivity of *Nicotiana tabacum* var. Samsum (tobacco) can be manipulated by transformation with complementary DNAs for glycerol-3-phosphate acyltransferases from squash and *Arabidopsis*. The genetic manipulation of fatty acid unsaturation is known to alter the chilling sensitivity of prokaryotes[10], and we have now demonstrated that it can also do so in higher plants.

Glycerol-3-phosphate acyltransferase cDNA from squash[11] and *Arabidopsis*[12], under the control of the cauliflower mosaic virus 35S constitutive promoter in the binary plasmid pBI-121, was introduced into tobacco plants. The cDNA encoding the full-length precursor of the enzyme from *Arabidopsis*, and containing the 5′ and 3′ noncoding regions[12], was inserted between the *Bam*H1 and *Sac*I sites of pBI-121, to form a plasmid designated pARA. The mature protein region of the cDNA for glycerol-3-phosphate acyltransferase from squash[11] was ligated with the transit region of the cDNA for the small subunit of pea Rubisco[13]. This construct was inserted between the *Bam*H1 and *Sac*I sites of pBI-121, to form a plasmid pSQ. These different constructs were introduced into *Agrobacterium tumefaciens* (LBA 4404) by electroporation and transformants of *A. tumefaciens* were selected by resistance to kanamycin and by DNA–DNA blot analysis for vector plasmids.

N. tabacum was transformed by the leaf-disk method[14] and the transformed calli were selected on Murashige–Skoog (MS) medium[15] containing kanamycin ($100\ \mu g\ ml^{-1}$) and claforan ($250\ \mu g\ ml^{-1}$). After shooting and rooting, transformants were grown at 25 °C on MS medium containing claforan ($250\ \mu g\ ml^{-1}$) in plastic boxes under fluorescent light ($50\ W\ m^{-2}$) for 16 h followed by 8 h dark. Fifteen independent kanamycin-resistant plants were obtained for each construct and their phosphatidylglycerol fatty acid composition analysed. Most plants