



Contingency and Determinism in Replicated Adaptive Radiations of Island Lizards

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both T1 and T2 transgenic plants (Fig. 4C). The amount of NPTII protein was not affected by infection in T3 plants, in which the *NPTII* transgene does not share homology with the CaMV promoter. The distribution of NPTII protein between dark green island and chlorotic vein border tissue of T1 transgenic plants (Fig. 4C) reflected that of GUS activity (Fig. 2B).

Suppression of the *NPTII* gene might have occurred through interference from the adjacent *GUS* gene. Alternatively, CaMV infection might result in host regulation of the 35S RNA promoter. Therefore, we tested the effects of CaMV infection on expression of the *GUS* transgene of the T3 construct (Fig. 1A), for which viral homology is limited to the CaMV 35S RNA promoter sequence. CaMV infection suppressed *GUS* expression in T3 transgenic plants with the same symptomatic pattern as that in T1 transgenic plants (Fig. 1C). However, silencing in T3 transgenic plants was not likely mediated by posttranscriptional mechanisms because the construct lacked viral RNA homology. Nuclear run-on experiments revealed that transcription of the T3 *GUS* transgene was inhibited in infected plants, despite concurrent transcription of the CaMV minichromosome (Fig. 4A). We suggest that transcriptional silencing of the 35S RNA promoter in the CaMV minichromosome does not occur in the presence of posttranscriptional silencing. However, transcriptional suppression of the CaMV 35S RNA promoter in the T3 construct suggests that viral transcription could potentially be down-regulated in those infections that do not result in recovery from symptoms as in *B. rapa*. Such regulation could explain the differential accumulation of CaMV in chlorotic and dark green tissue observed in the absence of posttranscriptional silencing (15).

Thus, plants respond to pathogen invasion by regulating pathogen gene expression, apparently at both transcriptional and posttranscriptional levels. Posttranscriptional suppression of viral genes results in posttranscriptional cosuppression of transgenes that share sequence homology with the virus. Sequences homologous to the viral promoter can be silenced at the transcriptional level. Posttranscriptional suppression of gene expression appears to take precedence over transcriptional regulation, possibly by preventing transcriptional suppression of the same gene, thereby linking cytoplasmic and nuclear gene regulatory mechanisms.

Most gene silencing phenomena that have been described in plants occur as a result of transformation with transgenes (5, 9). Gene silencing can also be elicited by viruses in the absence of transgenes (3, 4). It is not clear whether this response is an

tipathogenic or whether it is more broadly related to regulation of highly expressed genetic elements.

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- Nuclei were isolated as described (3). Incorporation of uridine 5'-[³²P]triphosphate (DuPont Biotechnology Systems) was determined by probing 1 μ g of the appropriate DNA samples immobilized as slots on Hybond-N⁺ membranes (Amersham).
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- Leaf disks (12 mm in diameter) were collected and treated as described [R. A. Jefferson, T. A. Kavanagh, M. W. Bevan, *EMBO J.* **6**, 3901 (1987)] for histochemical detection of GUS activity.
- In situ hybridization to detect CaMV was performed as described (15) with the same leaf disks as those used for histochemical detection of GUS activity.
- We thank N. Owen and C. Jones for producing transgenic lines, J. Jones for transformation constructs, and A. Lángara for advice on nuclear run-on assays. This work was supported by the U.K. Biotechnology and Biological Sciences Research Council and covered by license PHF 1491/982/34 of the Ministry of Agriculture, Fisheries and Food.

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Contingency and Determinism in Replicated Adaptive Radiations of Island Lizards

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The vagaries of history lead to the prediction that repeated instances of evolutionary diversification will lead to disparate outcomes even if starting conditions are similar. We tested this proposition by examining the evolutionary radiation of *Anolis* lizards on the four islands of the Greater Antilles. Morphometric analyses indicate that the same set of habitat specialists, termed ecomorphs, occurs on all four islands. Although these similar assemblages could result from a single evolutionary origin of each ecomorph, followed by dispersal or vicariance, phylogenetic analysis indicates that the ecomorphs originated independently on each island. Thus, adaptive radiation in similar environments can overcome historical contingencies to produce strikingly similar evolutionary outcomes.

The theory of historical contingency proposes that unique past events have a large influence on subsequent evolution (1–3). A corollary is that repeated occurrences of an evolutionary event would result in radically different outcomes (4). Indeed, faunas and

floras that have evolved in similar environments often exhibit more differences than similarities (5–7). These differences in evolutionary outcome probably result from clade-specific factors that cause taxa to respond to similar selective factors in different ways, as well as from unique historical events and subtle environmental differences in the different areas (2, 8). Here we show that such factors will not always lead to disparate outcomes.

Anolis lizards are a dominant element of the Caribbean fauna. On each of the islands of the Greater Antilles (Cuba, Hispaniola, Jamaica, and Puerto Rico), lizard assemblages are composed of species that differ in

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Table 1. Hypotheses tested with DNA sequence data. A significant result denotes rejection of the stated hypothesis. *D* is the difference in length between the most parsimonious tree (8889 steps) and the tree constrained to conform to the stated hypothesis. *T_s* is the test statistic for the Wilcoxon signed-ranks test. *n* is the number of characters that differed

in numbers of changes on the two trees. *Z* is the normal approximation when *n* > 100 (25). "Difference" is the difference in negative log likelihoods between the maximum likelihood tree (-ln L = 41,059.9) and the tree constrained to conform to the stated hypothesis. *t* is the Student's *t* test statistic.

Hypothesis	<i>D</i>	<i>T_s</i>	<i>n</i>	<i>Z</i>	Parsimony <i>P</i> value	Likelihood difference	<i>t</i>	Likelihood <i>P</i> value
Monophyly of ecomorph class								
Crown-giant	120	5,350	229	7.8	<0.001	424.2	7.5	<0.001
Grass-bush	165	17,647	339	6.2	<0.001	633.8	8.5	<0.001
Trunk	42	2,016	113	3.4	<0.001	110.0	2.5	0.014
Trunk-crown	201	7,921	289	9.1	<0.001	771.0	11.6	<0.001
Trunk-ground	175	22,927	382	6.3	<0.001	546.5	11.4	<0.001
Twig	99	12,882	270	4.2	<0.001	384.0	6.6	<0.001
Shortest tree with								
16 ecomorph transitions	5	2,706	106	0.4	0.683	51.1	1.4	0.171
15 ecomorph transitions	25	6,444	172	1.5	0.128	103.1	2.7	0.007
14 ecomorph transitions	48	7,803	198	2.5	0.011	212.3	4.5	<0.001

habitat use. The same set of "ecomorphs"—species specialized to use particular structural microhabitats—occurs on each island, except that two ecomorphs are absent from Jamaica and one from Puerto Rico (9).

We measured six morphometric characteristics that are closely linked to habitat use (10, 11) for members of each ecomorph class from each island to investigate whether the ecomorphs constitute objectively recognizable classes (12). Our analyses reveal distinct ecomorph classes; members of an ecomorph class are more similar to other members of that class from different islands than they are to members of different ecomorph classes from their own island (Fig. 1A) (13).

The presence of the same set of ecomorphs on each island suggests that either ecomorphs evolved only once and then, by colonization or vicariance, occupied all four islands, or that each ecomorph evolved independently on all four islands. Because six ecomorph classes exist (crown-giant, grass-bush, trunk, trunk-crown, trunk-ground, and twig; the classes are named for the microhabitat that constituent species normally use), the single-evolution hypothesis predicts that only five instances of the evolution of new ecomorphs have occurred (assuming that one ecomorph is ancestral). By contrast, the recurring evolution hypothesis (9) predicts that none of the ecomorph classes form a monophyletic group and that 17 to 19 evolutionary transitions between ecomorph classes have occurred (14).

Phylogenetic analysis based on mitochondrial DNA sequences (15, 16) for 55 species (17) indicates that, with two exceptions, members of the same ecomorph class from different islands are not closely related (Fig. 1B). Statistical analyses (18) indicate that none of the ecomorph classes constitutes a monophyletic group relative to members of the other classes and that at least 17 evolutionary transitions among

ecomorph classes have occurred (Table 1) (19, 20). Although similar sets of ecomorphs have evolved independently on

each island, the sequence by which they have evolved differs among islands (Fig. 1C) (21).

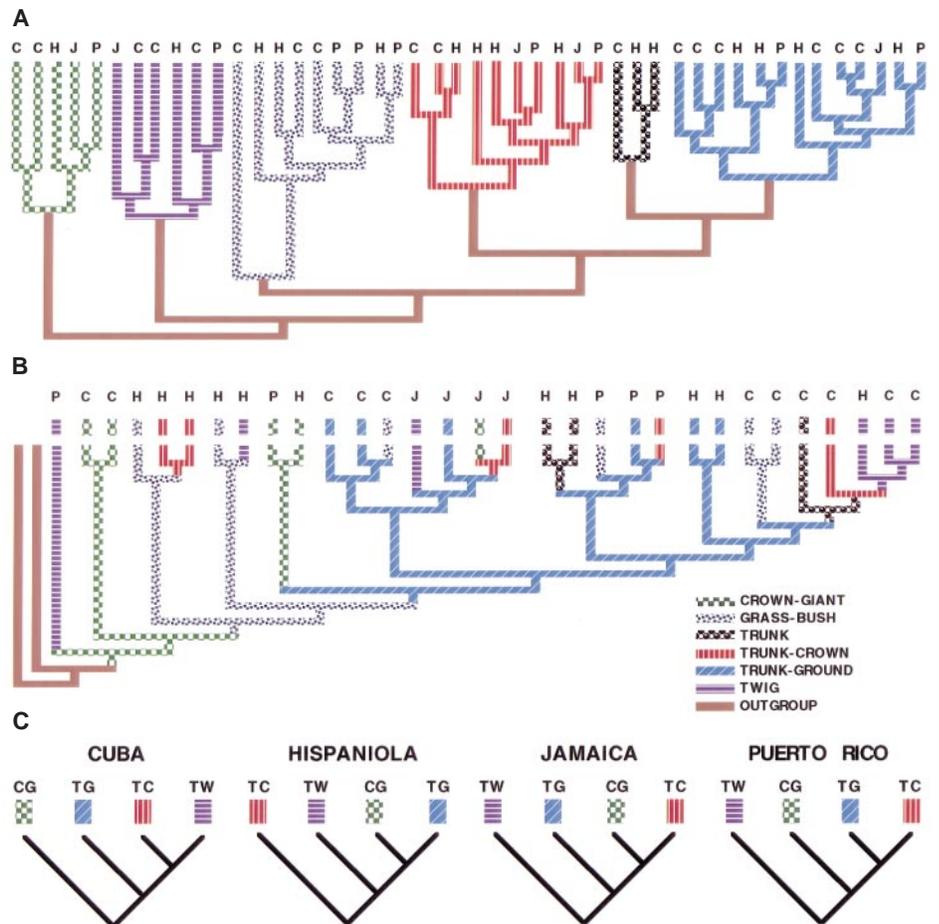


Fig. 1. (A) UPGMA phenogram showing that members of the same ecomorph class cluster in morphological space regardless of geographic affinities. Branch lengths are proportional to the distance separating species or clusters in morphological space. Letters indicate the island on which a species is found (C, Cuba; H, Hispaniola; J, Jamaica; P, Puerto Rico). The shading of the branches connecting the ecomorph classes has no significance. (B) The most parsimonious tree derived from the molecular data indicates frequent transitions among ecomorph classes. The lengths of the branches have no significance. (C) Topology of the four ecomorphs common to all islands, extracted for each island separately from the most parsimonious phylogeny.

One hypothesis to explain the repeated evolution of the same ecomorph types is that the diversity of morphological variants that can be produced by anoles is constrained to these ecomorphs. However, the existence of several Greater Antillean species, usually restricted to montane areas (9), and many mainland species (22) that are not members of any of the ecomorph classes shows that morphological diversification among anoles is not constrained to produce only members of these ecomorph classes. Rather, the recurring evolution of ecologically and morphologically similar species in these replicate adaptive radiations suggests that adaptation, rather than constraint, is responsible for the predictable evolutionary responses of *Anolis* lizards.

The phylogenetic analysis reveals only two cases in which an ecomorph has evolved more than once on a single island. Interspecific competition, which is intense among anoles (23) and may drive their adaptive radiation (9, 24), is probably responsible; once an ecomorph niche is filled on an island, other species are excluded from utilizing that niche. Thus, the importance of historical contingency depends on the frame of reference: Among islands, it has little discernible effect in that the same ecomorphs evolve on each island, whereas within each island, prior evolutionary events limit the options available to particular species and thus determine the directions in which evolution can proceed.

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- We measured live adult males of 46 species of Greater Antillean *Anolis*. Species were assigned to an ecomorph class a priori on the basis of morphological, behavioral, and habitat data. The following measurements were made of (usually) 10 to 15 individuals of each species: mass; number of subdigital lamellae on the second and third phalanges of pedal digit IV; and snout-to-vent length (svl), forelimb length, hindlimb length, and tail length. Mean values were used for each species. To remove the effects of body size, interspecific regressions were conducted for each variable against svl (all variables were ln transformed); residual values were used in subsequent analyses. To reduce the dimensionality of the data, a principal components (PC) analysis [H. Hotelling, *J. Educ. Psychol.* **24**, 417 (1933); H. H. Harman, *Modern Factor Analysis* (Univ. of Chicago Press, Chicago, ed. 3, 1976)] was conducted on the residual variables; ln svl was also included so that the analysis considered both size and shape. The first four PC axes accounted for 95.8% of the variance. We then used the unweighted paired group method using arithmetic averages (UPGMA) [R. R. Sokal and C. D. Michener, *Univ. Kans. Sci. Bull.* **38**, 1409 (1958)] to visualize the similarity relationships of species in a morphological space defined by these four PC axes.
- The UPGMA analysis indicates that members of each of the ecomorph classes cluster together in morphological space. UPGMA and other clustering methods sometimes distort similarity relationships [K. de Queiroz and D. A. Good, *Q. Rev. Biol.* **72**, 3 (1997)]; however, direct examination of the Euclidean distances (that is, the distance separating two species in the multivariate space determined by the four PC axes) confirms that every species is closer to a member of its own ecomorph class than it is to any member of any other ecomorph class. The probability that random assignment of species to ecomorph clusters will produce an arrangement in which the species are clustered by ecomorph class to the extent seen in the real data (that is, with no species out of place) is much less than 0.0001.
- The uncertainty depends on which ecomorph is ancestral. For example, if the ancestor were a trunk anole, then no instances of trunk anole evolution would be required, but four instances of trunk-ground anoles would be required. By contrast, if the ancestor were a trunk-ground anole, only two instances of trunk anole evolution would be required, because this ecomorph occurs only on two islands, whereas trunk-ground and most other ecomorphs occur on all four islands. These figures also assume, conservatively, that one ecomorph is ancestral to all of the others and that each ecomorph evolved only once per island.
- The amplification and sequencing of 1455 alignable base positions followed previously published procedures [J. R. Macey, A. Larson, N. B. Ananjeva, Z. Fang, T. J. Papenfuss, *Mol. Biol. Evol.* **14**, 91, (1997); J. R. Macey, A. Larson, N. B. Ananjeva, T. J. Papenfuss, *J. Mol. Evol.* **44**, 660 (1997)], except that annealing of primers for sequencing reactions was done at 53° to 61°C. Alignments of sequences encoding five transfer RNAs (Trp, Ala, Asn, Cys, and Tyr) were constructed on the basis of secondary structural models [Y. Kumazawa and M. Nishida, *ibid.* **37**, 380 (1993); J. R. Macey and A. Verma, *Mol. Phylogenet. Evol.* **7**, 272 (1997)]. All sequences have been deposited in GenBank.
- The most parsimonious phylogeny was estimated using PAUP* [D. L. Swofford, *Phylogenetic Analysis Using Parsimony*, version 4.0.0d60 (Smithsonian Institution, Washington, DC, 1997)] with 100 heuristic searches using random addition of sequences and tree bisection and reconnection branch swapping (TBR). To estimate the maximum-likelihood tree, we used the 493 trees within five steps of the most parsimonious tree as starting trees, performing a heuristic search with the TBR branch-swapping option in PAUP* with the following parameters: empirical base frequencies, a transition-transversion ratio of 2 (estimated from the most parsimonious tree as 2.049 and rounded down), and equal rates of substitution among sites. Although we did not include all ecomorph species in this analysis, almost all non-sampled species are closely related to a sampled member of the same ecomorph class from the same island. The two exceptions are rare species from Hispaniola (*A. darlingtoni* and *A. koopmani*) that may not be closely related to members of the same ecomorph class on that island [K. L. Burnell and S. B. Hedges, *Caribb. J. Sci.* **26**, 7 (1990)]. Hence, depending on the phylogenetic relationships of these two species, we may underestimate by two both the number of evolutionary origins of ecomorphs and the instances in which the same ecomorph evolved multiple times on the same island.
- Those 55 species included the outgroups *Diplolaemus darwini* and *Polychrus acutirostris*. Ecomorph species were a subset of those used in the morphometric analysis, with the addition of the Hispaniolan twig anole *A. sheplani*.
- To test a hypothesis of ecomorph monophyly, we used PAUP* to find the shortest tree with the constraint that an ecomorph class arose once and did not subsequently give rise to members of any other ecomorph class. The Wilcoxon signed-ranks test was then used to investigate whether the most parsimonious tree required significantly fewer changes than the tree constrained to show monophyly of an ecomorph class [A. Templeton, *Evolution* **37**, 221 (1983); A. Larson, in *Molecular Ecology and Evolution: Approaches and Applications*, B. Schierwater, B. Streit, G. P. Wagner, R. De Salle, Eds. (Birkhäuser, Basel, Switzerland, 1994), pp. 371–390]. Each ecomorph class was tested separately. We also determined the phylogeny that required the fewest instances of ecomorph evolution and was not significantly longer than the most parsimonious tree. PAUP* was used to find the shortest tree that included as sister taxa a pair of species (or clades) that were inferred to have evolved the same ecomorph independently on the most parsimonious tree. This constraint generally lowered the number of transitions among ecomorphs from 17 to 16; PAUP* searched all trees constrained in this manner and found the one requiring the fewest DNA changes. This tree was then compared to the most parsimonious tree with the use of the Wilcoxon signed-ranks test. Additional constraints were applied in the same manner to further reduce the number of origins of ecomorphs until all trees requiring fewer transitions were significantly longer than the most parsimonious tree. The same hypotheses were tested with the Kishino-Hasegawa test, comparing the maximum-likelihood tree to trees constrained as above [H. Kishino and M. Hasegawa, *J. Mol. Evol.* **29**, 170 (1989)].
- To estimate the number of evolutionary transitions among ecomorph classes, we reconstructed ancestral states using MacClade [W. P. Maddison and D. R. Maddison, *MacClade: Analysis of Phylogeny and Character Evolution*, version 3.0 (Sinauer, Sunderland, MA, 1992)]. Ecomorph state was treated as a partially ordered character, with the number of steps required for a transition from one ecomorph to another corresponding to the distance in morphological space between the centroids for the two ecomorph classes. Treating the states as unordered yielded the same estimate of the number of instances of ecomorph evolution inferred from this phylogeny. Nonecomorph species (21 species), most of which do not occur in the Greater Antilles, were not included in the ecomorph reconstructions. Thus, this method estimates the number of transitions among ecomorphs conservatively, because all ancestral nodes are assigned to one of the ecomorph classes.
- All trees within four steps of the most parsimonious tree also lead to the inference of 17 transitions, although reconstructions for particular ancestral nodes differ. Shortest trees requiring 15 or 16 transitions are not statistically significantly longer than the most parsimonious tree, but those requiring 14 transitions are (Table 1); the likelihood analysis yields similar results.
- To investigate whether the phylogenetic topology of ecomorph evolution was the same on all four islands, we examined the four ecomorphs present on all four islands. Figure 1C shows that each island exhibits a different topology. For each island separately, we examined each of the 15 possible topologies for four taxa to determine whether any topology is either the most parsimonious tree or is statistically indistinguishable from the most parsimonious tree for all four islands. If no topology met this criterion, then the data would indicate that ecomorph evolution did not occur in the same manner on all islands. For each island, we found the most

parsimonious tree conforming to each of the 15 possible phylogenetic topologies for the four ecomorphs (in the case of the two twig anoles on Hispaniola, we used *A. insolitus* because *A. sheplani* is the sister taxon of Cuban twig anoles and is nested within a clade of Cuban species) using the "backbone constraints" option in PAUP*, which constrains the relationships of a subset of the taxa but allows the remaining taxa to occur anywhere on the tree (that is, the subset of constrained taxa does not necessarily form a monophyletic group, but the relationships among these taxa must conform to the

constraint). We compared each of these trees to the most parsimonious tree (Fig. 1B) using the Wilcoxon signed-ranks test. In addition, we compared the maximum-likelihood tree with each constraint tree using the Kishino-Hasegawa test. Each of the 15 possible ecomorph topologies was rejected for at least one island. Hence, we conclude that the topology of ecomorph evolution differed among islands. In addition, when ancestral ecomorph states were reconstructed with parsimony, each island exhibited a different order of ecomorph evolution.

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Monoallelic Expression of the Interleukin-2 Locus

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The lymphokine interleukin-2 (IL-2) is responsible for autocrine cell cycle progression and regulation of immune responses. Uncontrolled secretion of IL-2 results in adverse reactions ranging from anergy, to aberrant T cell activation, to autoimmunity. With the use of fluorescent in situ hybridization and single-cell polymerase chain reaction in cells with different IL-2 alleles, IL-2 expression in mature thymocytes and T cells was found to be tightly controlled by monoallelic expression. Because IL-2 is encoded at a nonimprinted autosomal locus, this result represents an unusual regulatory mode for controlling the precise expression of a single gene.

IL-2 is a growth factor important in the regulation and differentiation of lymphocytes and natural killer cells (1). Produced by a subpopulation of activated T cells, IL-2 also plays a pivotal role in the generation of an adoptive immune response. Decreased secretion or the complete absence of IL-2 in humans is associated with primary and secondary immunodeficiencies (2). Mice ho-

mozygous for an IL-2 null mutation (IL-2^{-/-}) have a compromised immune system with alterations of both cellular and humoral functions (3). Overproduction of IL-2 results in an impaired immune response with autoimmunity, breaking of clonal anergy, and suppression of certain T cell functions (4). IL-2 expression, therefore, is firmly controlled by multiple signaling pathways emanating from the T cell receptor and antigen-independent coreceptors (5). These signals regulate the transcriptional control of ubiquitous and T cell-specific factors, which transactivate transcription of the gene encoding IL-2 in vivo through binding to the promoter and enhancer sequences using an all-or-nothing mechanism (5). Coreceptors also transduce signals that stabilize IL-2 mRNA (6).

The number of functional IL-2 alleles may also determine the amount of IL-2 produced. Therefore, we investigated whether T cells heterozygous for the IL-2 null mutation produce less IL-2 than wild-type T cells. We stimulated CD4⁺ T cells purified from wild-type and heterozygous mice. The amount of IL-2 produced by concanavalin A (Con A)-treated IL-2^{+/-} T cells was decreased by half when compared with that produced by T cells from wild-type mice (Fig. 1). As expected, Con A stimulation of IL-2^{-/-} T cells did not result in detectable IL-2 secretion.

Was each heterozygous CD4⁺ T cell pro-

ducing only half of the amount of IL-2 produced by wild-type cells, or were only half of the CD4⁺ T cells secreting amounts of IL-2 comparable with that secreted by wild-type T cells? Concurrent transcription from both (that is, the mutant and the wild-type) alleles of the IL-2 gene would lead to the first result, whereas the latter result would be obtained if allele-specific expression occurred from only one of the two copies of the IL-2 gene. To distinguish between these two mutually exclusive models, we determined IL-2 secretion at the single-cell level. Mature CD4⁺ thymocytes and CD4⁺ peripheral T cells were stimulated with Con A and subsequently stained for the presence of IL-2 (7). About half of the CD4⁺ T cells from 3- to 4-week-old heterozygous mice stained positively for IL-2 (Fig. 2, A and B, left). In agreement with these data, limiting dilution assays showed that the relative frequency of IL-2-secreting CD4⁺ T cells was diminished by a third to a half in heterozygous mice in comparison with

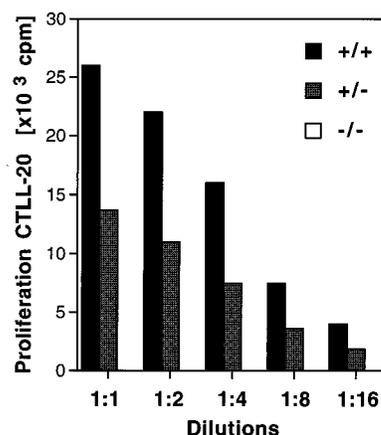


Fig. 1. The genotype of IL-2 mutant mice controls the amount of IL-2 secreted. IL-2 production in response to Con A stimulation. Purified T cells from heterozygous and homozygous IL-2 mutant mice and from wild-type mice were stimulated in vitro by Con A in RPMI 1640 medium (Gibco-BRL) supplemented with 10% fetal bovine serum (Sigma), penicillin, streptomycin, and 2-mercaptoethanol. After 24 hours in culture, serial dilutions of supernatant were assayed on 5×10^3 CTL-20 cells in the presence of mAb to IL-4 (11B11). Proliferation was measured by [³H]thymidine incorporation during the last 4 hours of a 24-hour assay. The graph is representative of three independent experiments and each experiment had less than 10% variability.

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