even be maintained in the face of parasitic interactions. For example, the caterpillars of at least three species of *Maculinea* (Table 1) live inside *Myrmica* ant nests to feed on the larval brood of their host ant species (*22*). Hence, this study points to the possibility that under selection for symbiotic associations, the calls of one insect species have evolved to attract other, distantly related insect species.

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7. The third to fifth (and final) instar caterpillars of *Thisbe irenea* bear three distinct sets of specialized organs for associating with ants: (i) paired, eversible organs which secrete food to ants on segment A-8; (ii) paired, eversible organs that presumably secrete a semiochemical to ants on segment T-3; and (iii) a pair of chitinized vibratory papillae on the anterior edge of segment T-1 that, in concert with oscillating the head in and out, beat most frequently when caterpillars are walking or are stressed. First and second instar caterpillars possess none of these organs. For a detailed account of *T. irenea* caterpillar biology see DeVries (*4*).
8. Calls were detected by placing caterpillars on a taut paper membrane sandwiched between 4-inch diameter plastic rings that had a particle velocity microphone touching the paper membrane. Calls were recorded on a Marantz PMD 420 cassette tape recorder, and subsequently analyzed with a Kay DSP Model 5500 Sonograph and a Data 6000 wave form analyzer. The microphone and amplifier was built to the specifications of H. C. Bennet-Clark [*J. Exp. Biol.* **108**, 459 (1984)].
9. Caterpillar calls were all of sufficiently low amplitude as to be detectable only by employment of the particle velocity microphone. As suggested by DeVries (*4*), it is likely that calls are produced when the shafts of the vibratory papillae drag across the granulations on the head when the caterpillar oscillates its head in and out.
10. Frequencies were measured for 76 individual pulses taken from 20 individual walking caterpillars: 15 fifth instar caterpillars (11 individuals, 4 pulses; 1 individual, 3 pulses; 2 individuals, 2 pulses; and 1 individual, 1 pulse), and five fourth instar caterpillars (4 pulses each).
11. Twenty-five individual caterpillars ranging from third to fifth instar had both vibratory papillae removed with finely pointed forceps. After subsequent testing with the particle velocity microphone, all caterpillars were allowed to molt, retested for sound production, and depending on the instar, had their vibratory papillae removed again. An additional five caterpillars, all fourth instars, had only a single vibratory papilla removed and were tested for their ability to produce sound.
12. To test if *T. irenea* caterpillar calls were attractive to ants, two sets of experiments were performed with field-collected caterpillars that were paired by instar and size before experimentation. One set was performed in an ambient temperature laboratory using captive ant colonies and potted plants ($n = 16$ pairs), and the other set was performed in the field with naturally occurring plants and ant colonies ($n = 35$ pairs). In each pair of caterpillars, one individual had the vibratory papillae removed, whereas the other individual retained them. Paired caterpillars were placed on individual plants where all caterpillars and ants had been removed immediately before experimentation. The pairs were then censused simultaneously for the number of ants tending each caterpillar at time intervals ranging from 1 to 12 hours, and each pair was left on the plant for 1 to 4 days of censuses. During the study each plant had only the paired caterpillars on it. All experiments used the same species of ant, *Ectatomma ruidum* (Ponerinae), and all caterpillar pairs were either fourth or fifth instars. The cumulative numbers of ants tending caterpillar pairs were compared by a Wilcoxon matched-pairs test.
13. Quantifying the attenuation of substrate-borne signals is problematical because signal attenuation varies with substrate, the dispersion rate, frequencies, and type of waves produced and the distance from signal [See M. Gogala (*20*)]. The natural substrate caterpillars and ants live on is a combination of leaves, petioles, stems, bark, and soil—all with varied physical properties. Yet on these types of substrates, insects may receive directional information from vibrational signals with frequencies similar to those found in caterpillar calls [see M. Gogala (*20*)]. Although there is little doubt that natural substrates have different transmission properties than the substrate on which calls were recorded (*8*), it is likely (but not yet tested) that ants could receive caterpillar calls from distances of 5 cm on a variety of substrates.
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Allometric Engineering: An Experimental Test of the Causes of Interpopulational Differences in Performance

BARRY SINERVO AND RAYMOND B. HUEY

Hatchling lizards (*Sceloporus occidentalis*) from a southern population are large and have high locomotor performance (speed and stamina) relative to hatchlings from northern populations. In order to determine whether differences in performance are an allometric consequence of interpopulation differences in size, yolk was removed from southern eggs, thereby producing miniaturized hatchlings equivalent in size to northern hatchlings. Miniaturized southern hatchlings no longer had higher speed than northern hatchlings, but maintained higher stamina. Interpopulation differences in speed but not in stamina are thus an allometric consequence of differences in egg size. Size manipulation adds an experimental dimension to allometric analyses.

EVER SINCE HUXLEY (*1*) FIRST DREW attention to the biological significance of relative size and shape, evolutionary and functional biologists have studied the allometric scaling of diverse morphological, physiological, and ecological traits (*2*). Allometric equations not only quantify the size dependence of a trait, but can also permit comparisons among individuals, populations, or species (*3, 4*) that differ in body size. Consequently, allometric analyses are often a key step in tests of hypotheses of trait evolution (*2–5*). Nevertheless, such analyses involve statistical, not experimental, adjustments of body size (*6*). Moreover, inferences about the proximate or mechanistic causes of dramatic differences in the intercept and slope of the allometry among taxa are risky, because many factors influence morphological and physiological traits (*7*).

Here we apply a novel method for experimentally manipulating body size, and we use this method to explore the mechanistic bases for interpopulational differences in the al-

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lometry of locomotor performance and morphology of hatchling lizards (*Sceloporus occidentalis*). Compared with hatchlings from northern populations (Oregon and Washington), hatchlings from a southern population (California) are large, have long hindlimbs, and have high burst speed and cruising stamina (Figs. 1 and 2). The high locomotor performances of southern hatchlings might be a mechanistic consequence of large body size (Fig. 1), of relatively long limbs (*8*) (Fig. 2A), or of other physiological or morphological differences. The involvement of these factors can potentially be tested at least three ways. First, the small northern hatchlings could be raced once they had grown to the size of (large) southern hatchlings, but this comparison would confound size and age. Second, analysis of covariance can often be used to determine if differences in a trait persist when body size is adjusted statistically, but such analyses may be misleading because causal factors responsible for trait divergence among populations may be different from the factors determining allometric scaling among individuals within a population. Third, between-population overlap in hatchling body size can be experimentally increased by removing yolk from the large southern eggs (*9*), thereby producing southern hatchlings that are similar in size to the small northern hatchlings (Fig. 1). If performances of size-matched hatchlings from the north and south are now comparable, then the observed interpopulational differences in performance would appear to be a simple allometric consequence of interpopulational differences in egg size. Alternatively, if locomotor performances of southern hatchlings are still high despite size standardization, then the observed interpopulational differences should be attributable to other evolved differences, not just to size.

We obtained eggs laid in captivity by females (southern California, central Oregon, and southern Washington) during May and June 1988 (*10*). To produce miniaturized hatchlings (cover and Fig. 1) from the southern population, we partially removed yolk from some of the freshly laid eggs of California females (*11*). A few eggs from each clutch were unmanipulated (control), and a few eggs were poked with the syringe but had no yolk removed (sham-manipulated) (*11*). Yolk removal produces miniaturized hatchlings because egg size and hatchling size scale with near isometry in both unmanipulated and manipulated eggs of *S. occidentalis* (*9*). The yolk-reduced, sham-manipulated and unmanipulated eggs resulted in a graded size series of hatchlings (see cover for a comparison of the resultant range of sizes of hatchlings from a single clutch). Additionally, many clutches were unmanipulated (*12*) and thus provide among-population comparative data. All eggs (hatchlings) were incubated (raised) under standardized conditions (*9*). When hatchlings reached 3 weeks of age, we measured their size, hindlimb span, and maximum burst speed on a laboratory racetrack (*13*). One to 2 weeks later, we measured their stamina on a slowly moving treadmill (*14*).

Some of the interpopulation differences in stamina persisted despite experimental reduction in body size (*15*) (Fig. 2B). Minia-

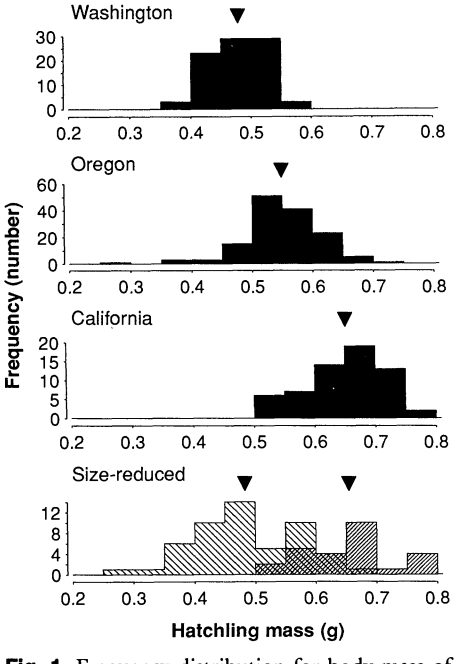

Fig. 1. Frequency distribution for body mass of unmanipulated hatchlings (solid bars, triangle indicates mean size) from Washington (n = 87), Oregon (n = 143), and California (n = 61) and for size-reduced hatchlings (light shading) from California (n = 78); dense shading indicates the size of unmanipulated sibs. Populations are significantly different with respect to unmanipulated hatchlings [ANOVA: $F(2,288) = 115.6$ $P < 0.001$]. Whereas the means of unmanipulated Washington and California hatchlings differ by more than three standard deviations, unmanipulated Washington hatchlings and size-reduced California hatchlings overlap broadly in size and the means are nearly identical.

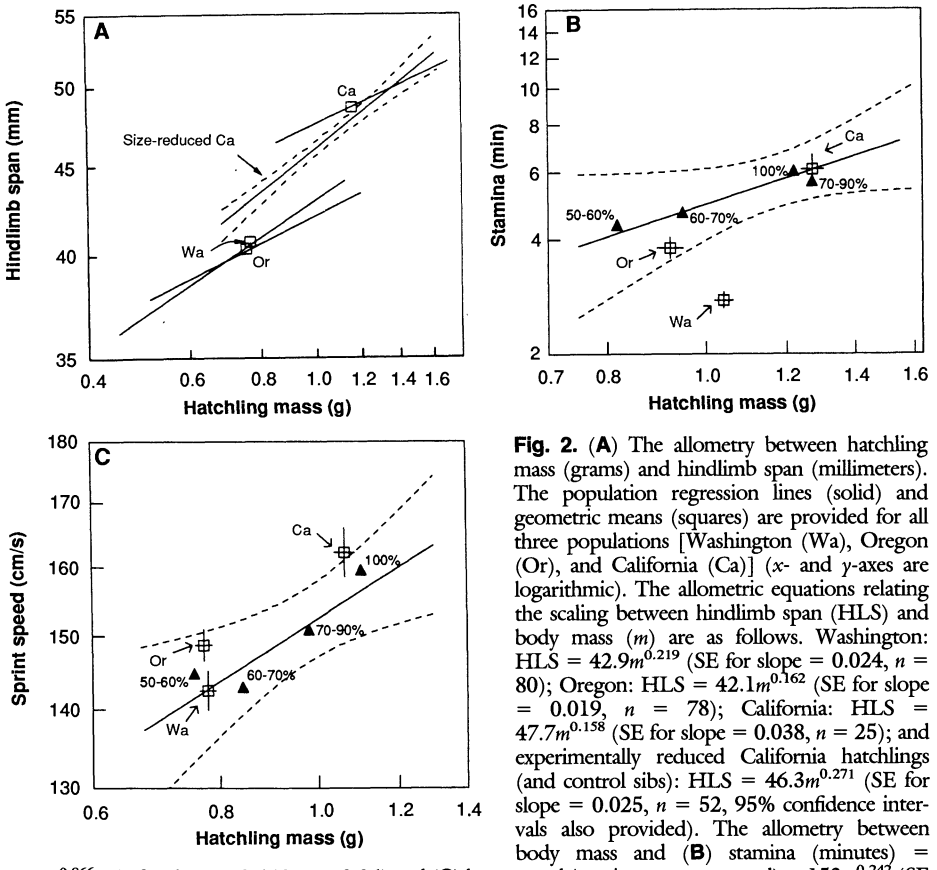

Fig. 2. (**A**) The allometry between hatchling mass (grams) and hindlimb span (millimeters). The population regression lines (solid) and geometric means (squares) are provided for all three populations [Washington (Wa), Oregon (Or), and California (Ca)] (x- and y-axes are logarithmic). The allometric equations relating the scaling between hindlimb span (HLS) and body mass (m) are as follows. Washington: $HLS = 42.9m^{0.219}$ (SE for slope = 0.024, n = 80); Oregon: $HLS = 42.1m^{0.162}$ (SE for slope = 0.019, n = 78); California: $HLS = 47.7m^{0.158}$ (SE for slope = 0.038, n = 25); and experimentally reduced California hatchlings (and control sibs): $HLS = 46.3m^{0.271}$ (SE for slope = 0.025, n = 52, 95% confidence intervals also provided). The allometry between body mass and (**B**) stamina (minutes) = $4.93m^{0.866}$ (SE for slope = 0.443, P = 0.06) and (**C**) burst speed (centimeters per second) = $152m^{0.242}$ (SE for slope = 0.094, $P < 0.01$); for miniaturized California hatchlings and their full-sized sibs (regression line with 95% confidence limits for a new mean). Squares represent the geometric (log-transformed) means (with SE) for mass and each performance trait for unmanipulated individuals from Washington (Wa) or Oregon (Or) and hatchlings from California (Ca). Triangles represent the geometric means pooled for various size classes of hatchlings resulting from varying degrees of size manipulation [100% (n = 7, unmanipulated; n = 15, sham-manipulated), 70 to 90% (n = 18), 60 to 70% (n = 12), and 50 to 60% (n = 5) of original egg mass remaining after yolk removal].

turized hatchlings from California still had much higher stamina than did those from Washington but not hatchlings from Oregon (*15*) (Fig. 2B). Thus, significant interpopulation differences in stamina of hatchlings, though in part an allometric consequence of differences in egg and hatchling size, are in large part due to other mechanistic causes, presumably those affecting aerobic capacity (*4, 16*).

In contrast, interpopulational differences in burst speed disappeared when body size was standardized and thus were causally related to interpopulational differences in egg size and thus hatchling size (Fig. 2C). Miniaturized southern hatchlings were no faster than were similarly sized northern hatchlings (*17*). Moreover, because miniaturized southern hatchlings from California still had longer legs (*18*) (Fig. 2A) but not faster speeds (Fig. 2C) relative to northern hatchlings, interpopulational differences in burst speed are unlikely to be purely a mechanistic consequence of differences in relative hindlimb length, despite presumed biomechanical links between these traits (*4, 8*).

Developmental manipulation of body size ("allometric engineering") adds to comparative biology a powerful new experimental dimension that can be used with diverse taxa (*9, 19*). Adult size can also be manipulated by the use of genetic engineering of the hormonal control of growth rate (*20*). However, this technique can currently be applied in only a few taxa. Size manipulation by either technique may allow comparisons between populations with limited overlap in body size (*21*), thereby permitting inferences on the proximate causes of trait evolution. Moreover, both techniques provide a direct experimental, not merely statistical, evaluation of the proximate influence of body size. For example, manipulation of hatchling size shows that interpopulational differences in sprint speed are probably an allometric consequence of interpopulational differences in egg size, but that interpopulational differences in stamina and morphology, though in part due to size, necessarily involve additional evolved factors (*4, 16, 22*). A comparison of experimental with traditional analysis of covariance (ANCOVA) analyses (*15, 17, 18*) demonstrates that purely statistical analyses of patterns can sometimes present a misleading portrait of the role of body size in populational differentiation in locomotor performance and morphology.

Of course, size manipulation (*9, 19, 20*) provides insights only into proximate—not ultimate—causes of interpopulational variation in traits. For example, our results do not suggest whether contemporary interpopulational patterns reflect natural selection in southern populations for large size or fast speed or both. Nevertheless, size manipulation does show that selection on size alone is unlikely to account for all the major interpopulational differences in locomotor performance (or the converse). Moreover, if the relative fitness of size-manipulated animals is measured in natural populations, some insights into the ultimate causes of interpopulational variation can be gained (*9, 23*).

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10. Near-term, gravid females were collected near Wrightwood, CA, on the east side of Table Mountain (altitude, 2230 m), near Terrebonne, OR (750 m), and near Lyle, WA (200 m).
11. Ten to 50% of total egg mass was removed with a 26° sterile syringe (n = 13 clutches). Incubation success of sham-manipulated controls and size-reduced eggs is similar, ~70%, as is post-hatching survival in the laboratory (*9*). Size reduction does not artifactually influence growth rate (*9*). Moreover, because the size-reduced lizards from California still have very high stamina, a stressful test of physiological capacity, size-reduction does not appear to hamper performance artifactually.
12. Washington (n = 7 clutches), Oregon (n = 16 clutches), and California (n = 9 clutches). The offspring from unmanipulated clutches obtained from California females were comparable to the sham-manipulated and unmanipulated controls obtained from experimentally manipulated clutches (*11*) with regard to hatchling size (Fig. 1), speed, and stamina (Fig. 2, B and C).
13. Mass (in grams), hindlimb span (millimeters measured between the fourth toe on each hind leg with hind legs stretched out laterally to either side). Individuals were raced six times (with 1-hour rest between races) by chasing them along a 1-m "racetrack." As a lizard ran down the racetrack, it interrupted photoreceptors spaced at 10-cm intervals, and the interval time between light stations was electronically sampled by a computer [R. B. Huey and P. E. Hertz, *J. Exp. Biol.* **110**, 113 (1984); D. B. Miles and R. G. Smith, *Funct. Ecol.* **1**, 281 (1987)]. Hatchlings were raced at 34°C, the optimal temperature for sprinting and the approximate average field body temperature [A. F. Bennett, *Anim. Behav.* **28**, 752 (1980); F. H. van Berkum, *Am. Nat.* **132**, 327 (1988); J. S. Tsuji, *Physiol. Zool.* **61**, 230 (1988)]. Maximum burst speed was the single fastest speed (centimeters per second) over any 20-cm interval from among all six trials.
14. To determine "cruising stamina" we placed hatchlings on a rubberized belt of a treadmill moving at 0.25 km/hour. When necessary to keep them running smoothly, we tapped them lightly on the tail and hindlimbs. Stamina was measured as the elapsed time (minutes) until a hatchling was exhausted, as verified by the loss of the righting response [F. H. van Berkum, R. B. Huey, J. S. Tsuji, T. Garland, Jr., *Funct. Ecol.* **3**, 97 (1989)].
15. Because the size of Oregon hatchlings and miniaturized hatchlings from California was similar, these populations could be compared without the confounding effects of size. This ANCOVA (log-transformed variables) indicated that the difference in stamina between Oregon and California hatchlings [0.25 log (min)] was not significant [$F(1,78)$ = 2.53, $P > 0.12$; covariate for size not significant (ns), $P > 0.60$]. ANCOVA comparing Washington hatchlings and experimentally miniaturized hatchlings from California indicated that the difference between populations (0.59) was significant [$F(1,86) = 20.98$, $P << 0.0001$; covariate for size ns, $P > 0.81$]. It is illuminating to compare the results obtained from this experimental analysis with a purely statistical analysis in which traditional ANCOVA of the data from the unmanipulated population samples was used. The traditional ANCOVA indicated that the difference between unmanipulated Oregon and California populations (0.41) was significant [$F(1,82) = 7.77$, $P < 0.007$; covariate for size ns, $P > 0.60$], as was the difference between Washington hatchlings and unmanipulated hatchlings from California [difference, 0.80; $F(1,90) = 48.82$, $P << 0.0001$; covariate for size ns, $P > 0.86$]. However, the population comparisons with traditional ANCOVAs overestimated the level of population differentiation, because a significant allometric effect of size on stamina was not detected in these analyses in contrast to analyses using the full range of size-manipulated hatchlings [$F(1,45) = 3.82$, $P = 0.06$, $n = 47$] (Fig. 2B). Moreover, the analysis using experimentally miniaturized hatchlings is not sensitive to problems arising from ANCOVA estimates of the allometric slope (*6*), because the size of comparison populations is closely matched in contrast to traditional ANCOVA population comparison.
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17. A comparison (ANCOVA of log-transformed data) of the burst speed of size-matched hatchlings from California with hatchlings from the two northern populations indicated that differences in each population were not significant [difference between California and Oregon, 0.018 log(m/s); $F(1,134)$ = 0.50, $P > 0.84$; covariate body mass ns, $P > 0.90$; difference between California and Washington, −0.006 log(m/s); $F(1,151) = 0.03$, $P > 0.86$; covariate for body mass ns, $P < 0.02$). In contrast, traditional ANCOVA using data from the unmanipulated population samples indicates that southern and northern hatchlings are different [difference between California and Oregon, 0.077; $F(1,134) = 4.663$, $P < 0.03$; covariate body mass, ns, $P > 0.90$; difference between California and Washington, 0.063; $F(1,151) = 3.178$, $P < 0.07$; covariate for body mass significant, $P < 0.03$]. However, the population comparisons using tradi-

tional ANCOVAs overestimated the level of population differentiation because a significant allometric effect of size on burst speed was detected inconsistently in these analyses in contrast to analyses based on the full range of size-manipulated hatchlings [$F(1,56) = 6.48$, $P = 0.01$, $n = 58$] (Fig. 2C).

18. Hindlimb span was shorter for northern hatchlings [ANCOVA: factor for population $F(2,199) = 118.6$, $P << 0.01$; covariate for size, $F(2,199) = 205.0$, $P << 0.01$, difference in slopes, ns). However, some of the difference between the hindlimb span of northern and southern populations arises from an effect of yolk mass on morphology [ANCOVA comparing unmanipulated California hatchlings with experimentally miniaturized hatchlings and their full-sized sibs indicates that yolk volume has a significant effect on morphology, slopes were significantly different between these groups, $F(1,73) = 5.95$, $P > 0.02$] (Fig. 2A).
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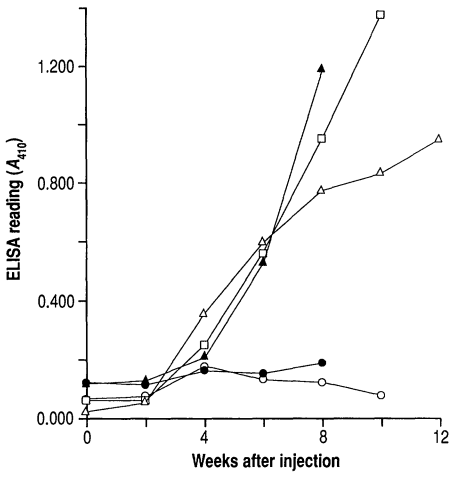

Fig. 1. Antibody responses in rhesus monkeys inoculated with SIVmac239 cloned virus. Portions of plasma from blood samples were frozen at −70°C on the weeks after inoculation and analyzed at a 1:20 dilution for antibodies to SIV by enzyme-linked immunosorbent assay (ELISA) as previously described (*6, 9, 19*). The five animals shown were inoculated with virus produced in macaque PBLs (*8*). The symbols used to identify the rhesus monkeys are ○, 316-85; ●, 452-87; □, 54-83; △, 326-87; and ▲, 124-79.

Induction of AIDS in Rhesus Monkeys by Molecularly Cloned Simian Immunodeficiency Virus

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Better understanding of the pathogenesis of acquired immunodeficiency syndrome (AIDS) would be greatly facilitated by a relevant animal model that uses molecularly cloned virus of defined sequence to induce the disease. Such a system would also be of great value for AIDS vaccine research. An infectious molecular clone of simian immunodeficiency virus (SIV) was identified that induces AIDS in common rhesus monkeys in a time frame suitable for laboratory investigation. These results provide another strong link in the chain of evidence for the viral etiology of AIDS. More importantly, they define a system for molecular dissection of the determinants of AIDS pathogenesis.

IDENTIFICATION OF THE GENETIC determinants of oncogenicity and tissue specificity of type C retroviruses has been achieved largely through the use of cloned DNA capable of yielding pathogenic virus (*1*). Human immunodeficiency virus (HIV), the causative agent of AIDS, is a member of the lentivirus subfamily of retroviruses. Although much has been learned about the molecular biology of HIV, systems for study of disease induction by molecularly cloned HIV have not been developed. In fact, there have been no previous reports of disease induction by a molecularly cloned lentivirus from any species.

The simian immunodeficiency viruses (SIVs) are nonhuman primate lentiviruses that are the closest known relatives of HIV-1 and HIV-2. They closely parallel their human counterparts in genetic organization and biological properties (*2*). Similarities between HIV and SIV include lentiviral morphology; tropism for CD4 lymphocytes and macrophages; extra genes called *tat, rev, vip, vpr*, and *nef* that other retroviruses do not have; use of the CD4 molecule for receptor; cytopathicity; and the ability to cause chronic disease after long-term persistent infection. Infection of common rhesus monkeys (*Macaca mulatta*) with some isolates of SIV results in AIDS and death in a time frame suitable for laboratory investigation (*3*). Features of the AIDS-like disease induced by SIV include CD4 lymphocyte depletion, opportunistic infections, severe weight loss, opportunistic neoplasms, and a multifocal granulomatous encephalitis. These are also features characteristic of HIV-induced disease in humans. The similarity in genomic organization, the extensive sequence homology, and the similarity in biological properties both in vitro and in vivo suggest that SIV systems are highly suitable for study of the mechanisms and determinants of HIV-induced disease.

In previous studies, three infectious molecular clones of SIV from macaque monkeys (SIVmac) were characterized (*4–6*). Of these three clones, SIVmac239 appeared to be most natural in that it grew best in primary cultures of macaque peripheral blood lymphocytes (PBLs) (*6*) and it retained a full-length 41-kD transmembrane protein rather than the truncated forms that result from growing SIV in human cells (*7*). We thus pursued in greater detail the pathogenic potential of this molecular clone.

SIVmac239-cloned DNA was transfected into primary macaque PBLs and into Hut 78 cells (a human $CD4^+$ T cell line) by a DEAE-dextran procedure, and stock virus was frozen for subsequent animal inoculations (*8*). To be useful for future mutagenesis experiments, it was important that we tested not only the original SIVmac239 lambda DNA clone but also plasmid subclones. We, and others, have experienced considerable difficulty in subcloning the full-length proviral DNA into plasmid vectors. We thus subcloned two segments of the DNA separately and then ligated them at their common restriction site before transfection (*8*).

Rhesus monkeys were inoculated with this cloned virus both at the New England Regional Primate Research Center (NERPRC) and at the California Regional Primate Research Center (CRPRC). In

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