a second set of standards by inserting three phased A:T tracts at different positions adjacent to the AP-1 site in the circular permutation analysis plasmids (*11*). The value $k = 1.06$ was determined from the best fit of Eq. 3 to the circular permutation function amplitudes of the standard probes.

15. The directed DNA bend angles induced by all of the complexes we investigated were smaller than the DNA flexure angles (Table 1). This difference between the DNA bend angle and the DNA flexure angle was not a result of an underestimate of the DNA bend angle by phasing analysis because the ranking of the complexes by DNA flexure angle and DNA bend angle was different. In addition, some complexes that induced little or no directed DNA bending [for example, Fos(139-211)-Jun] caused DNA flexure. We suggest that the larger DNA flexure angle is caused by other structural distortions such as an increase in DNA flexibility.
16. To determine the directed DNA bend angle, we calculated the best fit of a cosine function to the relative mobilities of phasing analysis complexes. The term "phasing function" refers to this best fit relation, and "phasing function amplitude" refers to the amplitude of this function. We have derived the relation between the phasing function amplitude (A_{PH}) and the directed DNA bend angle (α_B) from the dependence of electrophoretic mobility on end-to-end distance (Eq. 2). The ratio between the mobilities of two fragments of identical lengths but with different bend angles α_1 and α_2 at the middle is

$$\mu_1/\mu_2 = \cos(k\alpha_1/2)/\cos(k\alpha_2/2) \quad (4)$$

Tandem DNA bends are additive when placed in phase and cancel almost perfectly when placed out of phase [P. J. Hagerman, *Biochemistry* **24**, 7033 (1985); H.-S. Koo *et al.*, *Nature* **320**, 501 (1986)]. In the case of phasing analysis, the relation between maximum mobility, μ_{Max}, when the two bends counteract each other ($\alpha_1 = \alpha_B - \alpha_C$), and minimum mobility, μ_{Min}, when the two bends cooperate ($\alpha_2 = \alpha_B + \alpha_C$), is therefore

$$\mu_{Max}/\mu_{Min} = \cos[k(\alpha_B - \alpha_C)/2]/\cos[k(\alpha_B + \alpha_C)/2] \quad (5)$$

which can be resolved with standard trigonometrical relations to give

$$\tan(k\alpha_B/2) = \frac{\mu_{Max}/\mu_{Min} - 1}{(\mu_{Max}/\mu_{Min} + 1)\tan(k\alpha_C/2)} \quad (6)$$

Because μ_{Max} and μ_{Min} cannot be directly determined, we substitute $\mu_{Max}/\mu_{Min} = (1 + A_{PH}/2)/(1 - A_{PH}/2)$ to obtain

$$\tan(k\alpha_B/2) = \frac{A_{PH}/2}{\tan(k\alpha_C/2)} \quad (7)$$

Because the mobilities of bent DNA fragments depend on the end-to-end distance up to a bend angle of approximately 140°, and because the angle of the reference bend in these experiments is 54°, this function is expected to be valid up to a bend angle of approximately 90°.

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19. The Jun homodimer bends DNA in an orientation that is rotated 56° relative to the major groove–minor groove axis at the center of the AP-1 site. Our data indicate that the Jun homodimer subunits induce equivalent and symmetrical DNA bends. If the dimer interface is centered over the major groove (*8, 17*), the Jun homodimer can only bend DNA in an orientation parallel with the major groove–minor groove axis at the dimer interface. Therefore, the Jun homodimer must be displaced by 1.5 bp in order to align the observed orientation of bending with the major groove–minor groove axis.
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24. The structures of the Fos-Jun-DNA and Jun-DNA complexes predicted on the basis of the observed DNA trajectories were visualized with the SYBYL molecular graphics program. Since our data do not specify the distribution of the DNA bend among individual dinucleotides, we generated smooth bends by introducing equal wedges between each dinucleotide pair. Amino acid residues 136 to 200 in Fos and 254 to 318 in Jun were used to construct the models. These residues encompass the basic regions and leucine zippers of Fos and Jun, including the histidine residues at the COOH-terminal ends of the zippers that contribute to dimerization efficiency [D. R. Cohen and T. Cusran, *Oncogene* **5**, 929 (1990)]. The leucine zipper structure was modeled on the basis of standard coiled-coil conformation. Our data do not specify the orientation of Fos-Jun binding to the AP-1 site. However, in our model we favor the orientation in which Jun contacts the more stringently conserved half of the AP-1 site (*20*). The basic regions of Fos and Jun were modeled by starting with standard α helices and optimizing contacts with the major groove using both manual adjustments and local energy minimization programs (ANNEAL). The N cap in the Jun basic region was modeled on the basis of the conserved N cap geometry (*18*). Side chain conformations were not fully optimized. The atomic coordinates for this model will be provided on request.
25. We thank C. Cook for his assistance with the SYBYL molecular graphics program; C. Abate for providing Fos and Jun dimerization–DNA binding domain peptides for these experiments; and D. Nuss and C. Rosen for critical reading of the manuscript. T.K.K. is supported by a postdoctoral fellowship from The Helen Hay Whitney Foundation.

25 June 1991; accepted 9 September 1991

Genetic Mosaics in Strangler Fig Trees: Implications for Tropical Conservation

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Single trees of six species of strangler figs (*Ficus* spp., Moraceae) in Panama were found to be made up of multiple genotypes, presumably formed by the fusion of different individuals. The phenomenon is frequent enough that strangler fig populations will contain considerably more genetic variation than would be expected from the number of trees. How this cryptic variation affects populations depends on the flowering phenology of composite trees. If the genetically different portions of trees flower asynchronously, populations of pollinating wasps may be more resistant to low host population sizes than previously thought. If different portions flower synchronously, attempts to infer mating-system parameters from the parentage of fruit crops will be misleading. The fruiting of figs, which are considered a keystone species in tropical forests, is important for maintaining biodiversity but is also particularly susceptible to failure at small population sizes. It is therefore important to know both the number of trees and the number of genotypes in a population.

JANZEN (*1*) HAS MARSHALLED A COMpelling argument that figs (*Ficus* spp., Moraceae) possess sufficient biological peculiarities to render them fundamentally different from other tropical trees. Most of these distinctions arise from the fig's need for pollination by tiny, species-specific wasps (Agaonidae) that develop within the specialized inflorescences (*1–3*). The classical view [to which exceptions exist (*4*)] is that flowering and fruiting episodes are tightly synchronized: A tree releases a huge crop of pollen-bearing female wasps that must locate a conspecific tree that is at the proper (earlier) developmental stage to receive wasps; there, they can oviposit inside the inflorescences. Wasps of both sexes grow, pupate, and mate as the inflorescences develop; then the females leave to renew the cycle. This within-tree synchrony is coupled with between-tree asynchrony in flowering and fruiting, which is necessary to maintain populations both of pollinating wasps and, because some fig fruits are available all year, of fruit-eating vertebrates: "All the larger primates use figs heavily, as do procyonids, marsupials, guans, trumpeters, toucans, and many other birds . . . . Subtract figs from the ecosystem and one could expect to see it collapse" (*5*).

Figs are regarded as keystones (*5–8*) for conservation in many [if not all (*9*)] tropical forests, yet their flowering asynchrony renders them particularly vulnerable to forest reduction and fragmentation (*6, 10*). Although species of figs are numerous in most tropical forests, individuals are typically sparse (*1, 6*). Simulation models based on fig phenologies (*4, 11, 12*) suggest that about 100 trees may be necessary to maintain local populations of the wasps, leading to a proposal that 300 trees might be considered a minimum viable population size (*6*); wasps die within a few days if no tree is receptive when they are released, and fig

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fruits cannot develop unless wasps are available when the flowers are ready. Thus, the maintenance of a fig population is peculiarly dependent on the number of individuals, and population estimates will be critical in conservation decisions.

We show that for a large group of figs, the number of genetic individuals in a population may substantially exceed the number of trees, because many trees are composed of genetically different, fused individuals.

Of the approximately 750 species of figs worldwide, many are hemiepiphytic "stranglers," especially in the subgenus *Urostigma* (280 species) (*13–15*). Their seeds germinate in humus-filled crotches of host trees. As shoots grow upward, roots grow down and around the bole of the host, crossing and eventually fusing with each other to form a unified woody lattice ensheathing the host. Often, the host is killed, leaving a freestanding, hollow fig tree (*14*).

Because the defecatory habits of seed dispersers should commonly result in multiple seedlings colonizing a host, a question arises as to whether different strangler individuals fuse with each other when their roots make contact. Putz and Holbrook (*14*) briefly mention that such interindividual grafts ("allofusions") exist in nature, but their one-sentence account gives neither morphological details nor data on fusion frequency. It is extremely difficult to detect allofusions with certainty by simple examinations of older material because continuing woody growth soon obliterates the underlying structures. To determine whether allofusions are frequent and persistent enough to be of importance in natural populations, indirect approaches are needed.

Hamrick and Murawski (*16*) compared allozyme variation in rare and common rainforest trees on Barro Colorado Islands (BCI), Panama. Sixteen common species had a mean genetic diversity of 0.183. Of the rare species (those with fewer than 25 individuals in a 50-ha plot) three strangler figs had a mean diversity of 0.220, whereas the mean diversity of the other 13 rare species was 0.123. This pattern is consistent with the idea that some fig individuals might be made up of several genotypes, although variation within trees was not examined directly.

In November 1989 we analyzed allozyme variation within trees of six species of strangler figs on BCI and the surrounding peninsulas. The only criteria for choosing trees were large size and accessibility of multiple branches by boat. In one case, we sampled an individual that had previously been noted to have asynchronously flowering branches. We selected one young leaf from each of several branches of each tree, carefully ascertaining that all the branches were indeed connected. The leaves were freeze-dried within 2 days, then stored frozen until May 1990, when they were subjected to starch-gel electrophoresis for 18 enzyme systems.

Table 1 summarizes the allelic differences detected within apparent "individuals." Even though several trees were only represented by two or three leaves, 13 of the 14 sampled trees (and all of the species) showed detectable genetic differentiation among branches. These 13 trees included at least 45 genetic individuals. In seven trees, each sampled branch was different. Of all genotypic distinctions within plants, 20 were based on a single locus, but 25, 19, and 9 distinctions were based on 2, 3, and 4 loci, respectively. Given that any such search for allozyme differences must underestimate the true number of genotypes present, we expect that most large stranglers will be made up of multiple, fused genotypes. Somatic mutation could contribute to the genetic diversity we observed, but given that branches often differ at more than one locus, and given the figs' extreme predilection for root fusions, we expect that post-germination fusion is the dominant cause of the mosaicism.

Table 1. Summary of allozyme differences detected among branches of *Ficus* spp. trees. Within trees, each leaf represents a different branch. Leaves were surveyed for 13 enzymes (fluorescent esterase, leucine aminopeptidase, isocitrate dehydrogenase, phosphoglucomutase, phosphoglucoisomerase, glutamate oxaloacetate transaminase, aldolase, diaphorase, menadione reductase, triosephosphate isomerase, malate dehydrogenase, 6-phosphogluconate dehydrogenase, shikimate dehydrogenase), yielding 18 usable loci. The last column considers the mean number of loci that differ, averaged across all pairwise combinations of genotypes within a plant. Tree 2 of species "near *trigonata*" was specifically chosen because branches were fruiting asynchronously. This taxon is a strangler fig that is otherwise similar to the free-standing *F. trigonata* (*20*).

Species	Plant	Leaves (*n*)	Genotypes (*n*)	Loci (*n*)
citrifolia	1	4	3	2.0
	2	4	1	—
	3	3	3	2.7
	4	8	5	2.4
	5	8	8	2.4
colubrinii	1	2	2	3.0
costaricana	1	2	2	1.0
obtusifolia	1	2	2	1.0
	2	3	3	3.0
	3	5	4	2.7
perforata	1	2	2	1.0
	2	11	5	1.4
Near *trigonata*	1	4	3	2.7
	2	9	2	1.0
Total	14	67	45	

In figs, it is probably of mutual benefit for two young seedlings to fuse: Both gain mechanical stability, and by hastening the host's demise, both may receive more light and soil resources. A large fig would, however, receive little benefit from fusing with a smaller one; Titus *et al.* (*17*) state that fig seeds are usually incapable of germinating on fig host trees, although their search for an allelopathic effect yielded negative results.

The consequences of genetic mosaicism for figs' population biology depend on the possibilities for gene exchange, which in turn depend on flowering phenology. The degree of physiological integration between genetically different portions of a composite fig tree is unknown. A single trial of drawing colored water through the vasculature of a young, three-rooted, three-shooted *F. citrifolia* suggested that each root had vascular connections to one shoot only—that is, that there was little scope for translocation of nutrients or hormones. Possibly, different branches of composite individuals function independently, sharing only structural connections. In such a composite, branches might flower and fruit asynchronously. Thus, outcross pollinations could occur within the crown of a single tree, wasp populations might be more easily maintained and, indirectly, frugivores would benefit from more consistent fruit production. It is uncertain whether such asynchronies are common. Certainly, some species of figs characteristically flower asynchronously [for example, *F. aurea* in Florida (*4, 18*)], but in these cases, asynchrony occurs within single branches, which is presumably not a result of the kind of fusions we envision. Although there have been several studies of fig phenologies, few if any have tracked particular branches, and "within-crown fruiting synchrony has not been carefully studied in any of the Barro Colorado Island *Ficus*" (*3*).

The disparate genotypes of composite trees might, however, come into phenological synchrony. Then, neither pollination nor fruit set would be enhanced by the hidden genetic diversity; however, the greater diversity itself could be important for the conservation of the species. Synchronous reproduction of the genotypes within a tree could also complicate the estimation of minimum viable populations by indirect means. The above models require a minimum number of trees within the effective dispersal distance of the wasps. Wasp dispersal capabilities are hard to measure, but could be estimated indirectly by analyzing the paternity of a crop of fig seeds. For a population of a given size, a predictable number of trees should be contributing pollen to one fruit crop. Thus, "fathers per fruit crop" could serve as a substitute variable for "population

size," and could also identify a minimum viable population size. If paternity analysis of a fruit crop indicated this number of different fathers, it would suggest that the population was safe. With genetic mosaicism at the levels we have shown, however, that number of fathers could be coming from a single tree, and despite the high genetic diversity, the population could have far fewer trees than necessary for phenological continuity and wasp maintenance.

Thus, the basic biology of strangler figs may be even more peculiar than has been supposed. These plants might profitably be compared to some of the sedentary invertebrates that often show fusions (*19*). Further investigation of allofusion frequency, allorecognition specificity, wood anatomy, physiological integration, and reproductive synchrony should improve conservation programs for this disproportionately important (*1, 5, 6*) component of tropical forests.

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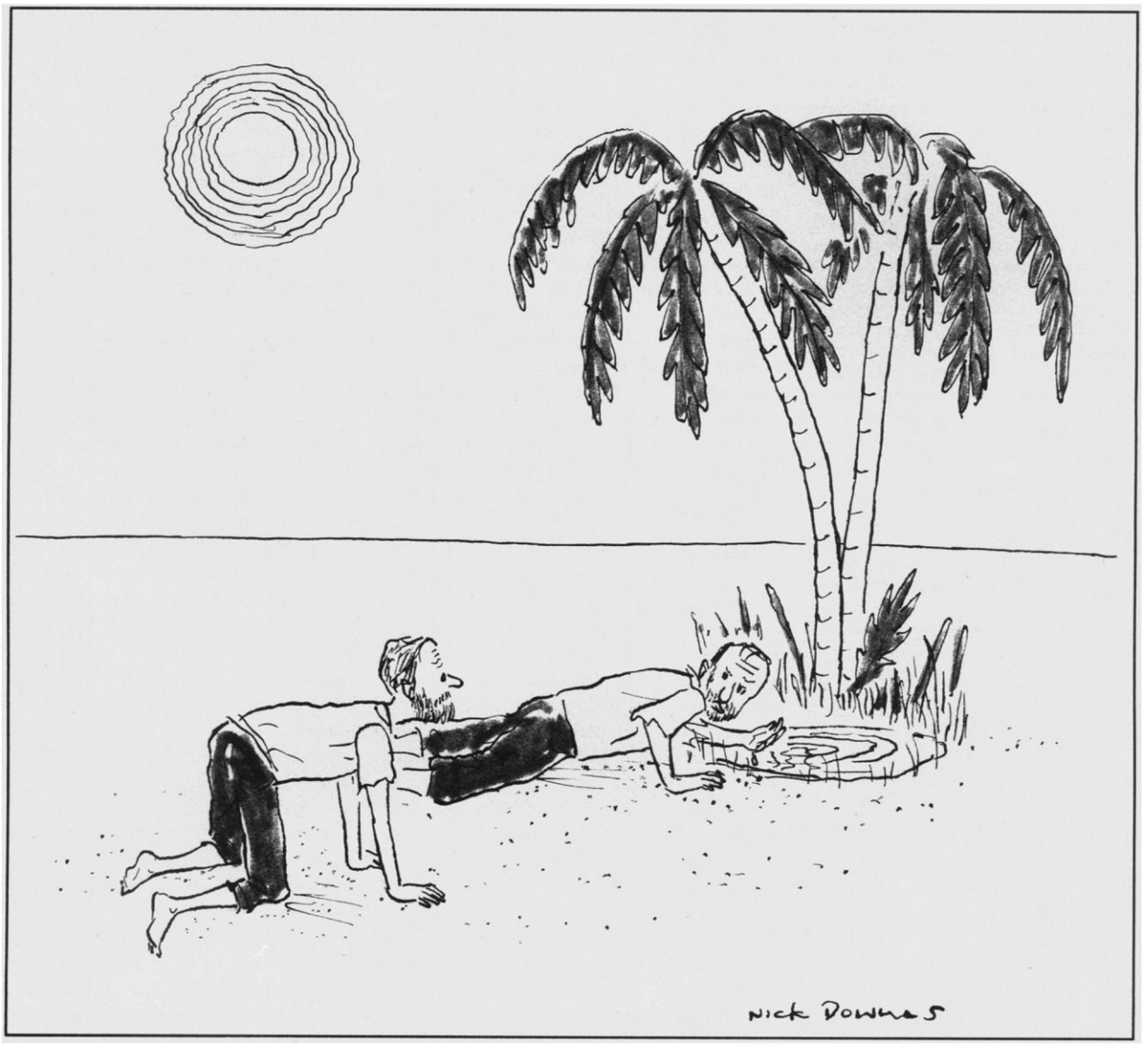
21. We thank D. Snyderman for assistance, F. E. Putz for inspiring the study, and D. Windsor and the Smithsonian Tropical Research Institute for hospitality and funding. Numerous colleagues responded to our queries for information or commented on the manuscript, especially J. Bronstein, M. Geber, C. Augspurger, P. Raven, J. Kress, and J. Kuijt. J.D.T. and J.L.H. were additionally supported by NSF. Contribution 797 in Ecology and Evolution, SUNY–Stony Brook.

28 May 1991; accepted 12 September 1991



"Look—who* cares *what the nitrate level is?"