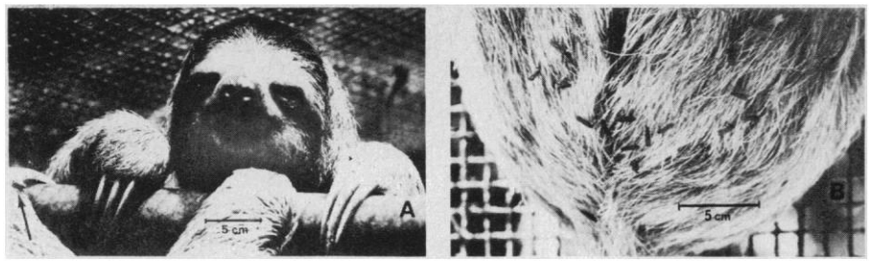

Fig. 1. (A) The three-toed sloth, *Bradypus infuscatus*, host of *Cryptoses choloepi*. There is a moth on the sloth's right foot (arrow). (B) A population of moths on the back of a sloth.

into the forest canopy where they find a sloth, mate, and continue the life cycle.

Sloths are common in the rain forests of Panama; they occur at population densities of about ten sloths per hectare on BCI (*4*) and at about six per hectare on Cerro Azul (*5*). Thus there would be an ample opportunity for a newly emerged moth to find a sloth by flying a short distance up and through the forest canopy. It is unlikely that newly emerged moths colonize the sloths which their parents infested, as sloths regularly move from tree to tree. Most two-toed sloths studied on BCI changed trees on successive days, while three-toed sloths were in the same tree on successive days about 40 percent of the time (*8*).

To study the behavior of adult *Cryptoses* on their hosts, we observed the movements of moths on captive and wild sloths. Particular attention was paid to possible feeding at nasal and lachrymal secretions, a behavior that is known for many species of tropical "eye moths" (*9*). The proboscis of *Cryptoses* is very short, but moths readily imbibed water in the laboratory, and this increased their longevity. While moths were active on the surface of the sloth, especially when the latter was moving, no instances of feeding at the eyes or nose were seen. Adult moths may feed on secretions of the sebaceous glands at the base of the host's hairs, or perhaps on rainwater trapped in the dense coat of the sloth. This water might be enriched by dissolved skin secretions and algal by-products.

As the presence of *Cryptoses* does not appear to have a detrimental effect on three-toed sloths, the relation between the moth and the sloth is most properly described as a phoretic rather than a parasitic one. *Cryptoses* benefits from this association through (i) the enhancement of oviposition-site location (that is, being carried by the sloth to the next fresh dung pile), (ii) the use of the sloth as a refuge from avian predators, and, perhaps, (iii) the enhancement of its diet with secretions of the host or associated algae. Similar phoretic associations between coprophagous insects and dung-producing mammals have been found in the Coleoptera (*10*) and Diptera (*11*), and may represent an incipient stage in the evolution of ectoparasitism.

JEFFREY K. WAAGE*
Princeton University, Princeton, New Jersey 08540
G. GENE MONTGOMERY†
National Zoological Park, Smithsonian Institution, Washington, D.C. 20009

References and Notes

1. R. Askew, *Parasitic Insects* (American Elsevier, New York, 1971), pp. 93 and 94; J. Baer, *Ecology of Animal Parasites* (Univ. of Illinois Press, Urbana, 1952), p. 90; J. Bourgogne, in *Traite de Zoologie*, P.-P. Grasse, Ed. (Masson et Cie, Paris, 1951), vol. 10, p. 330; T. Cameron, *Parasites and Parasitism* (Methuen, London, 1956), p. 190; M. Hering, *Biologie der Schmetterlinge* (Springer-Verlag, Berlin, 1926), pp. 74 and 130; W. Linsenmaier, *Insects of the World* (McGraw-Hill, New York, 1972), p. 228.
2. R. Casebeer and C. Hogue [*Nat. Hist.* **75**, 40 (1966)] examined sloths infested with *Cryptoses* in Costa Rica. The South American sloth moths, *Bradypodicola hahneli* Spuler and *Bradypophila garbei* von Ihering, have not been studied.
3. Tree-climber's spikes were used to climb trees in which sloths were seen. Extendable aluminum poles fitted with either a noose, a saw, or a pruning attachment were used to capture the sloth or drop it to a helper below. After capture, sloths were immediately put in large cloth bags. Few moths were lost during capture. Moths were later removed from sloths with an aspirator under a tent of mosquito netting. Moths came to the surface of the fur when disturbed; and the docility of the sloths permitted a thorough investigation to ensure that no moths were missed.
4. G. G. Montgomery and M. E. Sunquist, in *Tropical Ecological Systems: Trends in Terrestrial and Aquatic Research*, F. B. Golley and E. Medina, Eds. (Springer-Verlag, New York, 1975).
5. Dung was collected on Cerro Azul, Panama, at about 1500-m elevation during a census of sloths taken as a part of a study of sloths as reservoirs of yellow fever (G. Montgomery and C. Seymour, unpublished; C. Seymour *et al.*, in preparation). Dung was incubated in petri dishes placed outdoors in shade on Naos Island, Panama Canal Zone, at sea level. Counts of total numbers of moths that might have emerged and determination of sex ratios were not possible because the dung was inadvertently allowed to dry.
6. Moths were collected by H. Wolda at a light trap in Las Cumbres, Panama, on a year-round basis. The insects were pooled to estimate sex ratios.
7. G. Montgomery, W. Cochran, M. Sunquist, *J. Wildl. Manage.* **37**, 33 (1973).
8. M. Sunquist and G. Montgomery, *J. Mammal.* (1973), p. 54.
9. W. Buttiker, *Mitt. Schweiz. Entomol. Ges.* **39**, 151 (1967); E. Reid, *Proc. R. Ent. Soc. London Ser. B* **23**, 201 (1954).
10. Several dung beetles are known to infest sloths [G. Arrow, *Ann. Mag. Nat. Hist.* **63**, 385 (1933)] and kangaroos [E. Mathews, *Aust. J. Zool. Supp. Ser.* **9**, 3 (1972)], but only the beetles that infest kangaroos are known to feed on the host's dung.
11. The adults of certain muscid flies associate with cattle, on which some species actually feed. See O. Hammer, *Biological and Ecological Investigations on Flies Associated with Pasturing Cattle and Their Excrement* (Bianco Lunos Bogtrykkeri A/S, Copenhagen, 1941).
12. This research was conducted at the Smithsonian Tropical Research Institute. We thank M. Busch, H. Wolda, and N. Smith for their help. J.K.W. was supported by a fellowship from the Noble Foundation.
* Present address: Imperial College Field Station, Silwood Park, Ascot, Berkshire SL5 7PY, England.
† Present address: Smithsonian Tropical Research Institute, Box 2072, Balboa, Panama Canal Zone.

5 March 1976; revised 27 April 1976

Restriction Endonuclease Analysis of Mitochondrial DNA from Normal and Texas Cytoplasmic Male-Sterile Maize

Abstract. *Mitochondrial DNA from normal and cytoplasmic male-sterile maize was digested with restriction endonucleases RI from* Escherichia coli *or dIII from* Hemophilus influenzae. *Electrophoresis of resulting fragments revealed distinctions between the two cytoplasmic types. These distinctions suggest that factors responsible for cytoplasmic male sterility are located in the mitochondrial DNA, and that the mitochondrial genome is not inherited paternally.*

Although cytoplasmic (extrachromosomally inherited) male sterility of maize was first reported more than 40 years ago (*1*), the location of the factors conditioning this trait remains unknown. At least five different cytoplasmic male sterility types have been authenticated and a number of others have been reported, although their uniqueness has not been verified (*2*). The most widely known and studied is the Texas type male-sterile cytoplasm (cms T). Prior to 1970 it was extensively used to avoid detasseling in the production of hybrid seed corn (*3*). The occurrence of two leaf diseases in epidemic proportions (southern corn leaf blight and yellow leaf blight) on maize lines carrying cms T (*4*) forced the hybrid seed industry to abandon use of cms T in seed production. Recent studies have shown differences in the response of mitochondria from maize with normal and Texas cytoplasms when challenged by toxins (*5*) produced by race T of *Bipolaris maydis* (Nisikato) Shoemaker (southern corn leaf blight) and *Phyllos-*

ticta maydis Arny and Nelson (yellow leaf blight). Since these studies suggest the involvement of mitochondria, mitochondrial DNA (mtDNA) must be considered as a possible site of genetic factors responsible for traits associated with cms T.

Traditional methods of genetic analysis for nuclear genes are generally not available for studying cytoplasmically inherited traits of higher plants. An alternative approach lies in characterizing the organelle DNA's and attempting to relate them to the cytoplasmically inherited traits. When a homogenous DNA is digested by a site-specific restriction endonuclease, a characteristic array of fragments is generated. If the DNA is of low complexity, fractionation of the restriction fragments by gel electrophoresis results in a characteristic fragment pattern. This pattern can serve as a fingerprint of the original DNA molecule in a manner analogous to the tryptic fingerprints of protein (*6*).

We have isolated mtDNA from maize with normal (fertile) and Texas male-sterile cytoplasm. When the mtDNA's from the two cytoplasms were subjected to restriction enzyme digestion and fragment analysis, the patterns were readily distinguishable. This distinction in fragment patterns has been demonstrated to be consistent by examining mtDNA from normal and Texas cytoplasms in different genetic (nuclear) backgrounds. These results establish an association between mtDNA and the Texas type male-sterile cytoplasm.

Mitochondria were isolated from etiolated maize coleoptiles as described (*7*). Mitochondria were treated with deoxyribonuclease I prior to lysis with 1 percent Sarkosyl. The DNA from such preparations was centrifuged in cesium chloride–ethidium bromide gradients (*8*), and mtDNA was obtained by collecting upper (nicked) or lower (supercoiled) DNA bands. The authenticity of the nicked and supercoiled DNA was verified by electron microscopy (*9*). The DNA's were extracted with isopropyl alcohol to remove ethidium bromide and dialyzed against the buffers used in the restriction enzyme treatment. Occasionally the DNA was pelleted at 132,000*g* for 12 hours, the supernatant discarded, and the DNA dissolved in the restriction buffer. Upper and lower bands were used for digestion; no differences could be detected in subsequent fragment patterns and hence upper and lower bands were occasionally combined. The mtDNA's were then digested with restriction endonuclease RI from *Escherichia coli* (*10*) or dIII from *Hemophilus influenzae* (*11*), and the resultant DNA fragments were separated by electrophoresis in 0.7 or 1.0 percent agarose gels (*12*). Restriction fragments were visualized by fluorescence after staining with ethidium bromide (*13*).

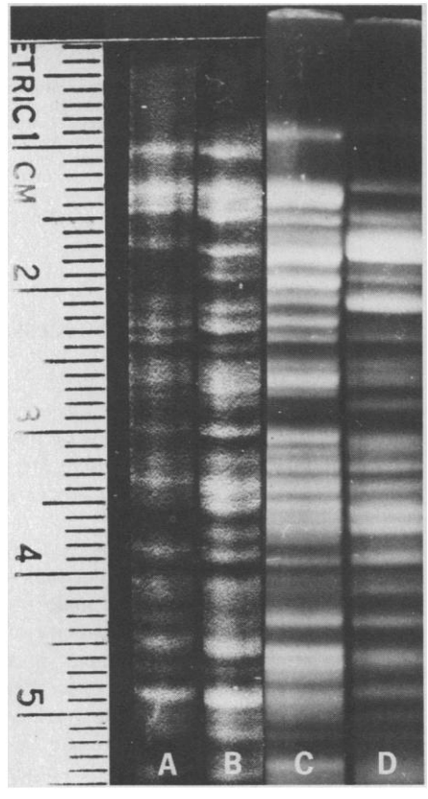

Fig. 1. Agarose gel electrophoretic patterns of maize mtDNA's after digestion with restriction endonuclease RI from *E. coli* (A and B) or dIII from *H. influenzae* (C and D). The maize lines and crosses used as sources of mtDNA were as follows: (A) NC7 × T204(N); (B) NC7 × T204(T); (C) W64A(N); and (D) W64A(T). Only the first 5.5 cm of the 12- or 16-cm gels is shown; most of the pattern alterations occurred in this portion of the gel.

The patterns obtained from digestion with dIII revealed about 50 bands, while RI produced about 40 bands. The fragment patterns in the upper 5.5 cm of the gels (Fig. 1) show that the normal and cms T fingerprints are different. Although the cleavage patterns from the two restriction endonucleases are not similar, both nucleases generated fragment distinctions between the mtDNA's of normal and Texas cytoplasms. We examined mtDNA from normal cytoplasm of three inbreds and two single crosses and found all the fragment patterns to be indistinguishable when cleaved by the same endonuclease. Similarly mtDNA from the same three inbreds and two single crosses but with cms T cytoplasm resulted in indistinguishable patterns after treatment with the same restriction endonuclease (*14*). Although the two types of cytoplasm yield readily distinguishable fragment patterns, there is considerable homology in the DNA's as evidenced by the large number of common bands.

Although these experiments were designed to localize the factors responsible for cytoplasmically inherited traits, they also provide unique evidence of uniparental inheritance of the mitochondrial genomes. Similar experiments indicated that horse and donkey mtDNA were not inherited paternally (*15*). In our study, we had hybrids in which the male parent contained normal cytoplasm and the female, Texas cytoplasm. Since the mtDNA's of the two cytoplasms are distinguishable, the parental DNA's are marked. The dIII digestion pattern of normal mtDNA contains several fragments (Fig. 1, 4.5 to 5.1 cm) which are not present in the cms T pattern. These fragments effectively mark the male parent, and we have repeatedly been unable to observe these specific fragments in the progeny. The pattern of the mtDNA of the cross was always that of the female parent. The same conclusion was found for cms T inbred lines which must be maintained by repeated crossing with male-fertile (normal cytoplasm) lines.

The purity of our mtDNA is an important concern. We examined our mtDNA by buoyant density determinations in neutral cesium chloride. Maize mtDNA is resolved as a single band with a density of 1.706 g/cm^3, which is typical of other higher plant mtDNA's (*16*). This density is different from that of maize nuclear or chloroplast DNA's (*17*). We determined the buoyant density of the upper and lower DNA bands obtained from cesium chloride–ethidium bromide preparative gradients and their densities were in agreement with the expected value for maize mtDNA. In our preparations the presence of sufficient nuclear DNA to influence the resultant patterns can be discounted because fragment patterns from upper and lower DNA bands were indistinguishable by our techniques. Furthermore, when maize nuclear DNA is digested by endonuclease and electrophoretically separated, no discrete bands are observed because of its complexity. Nonetheless, we cannot unequivocally state that our preparations do not contain an alien DNA. The possibility of a DNA-containing virus or viruslike agent in our maize lines is difficult to eliminate.

These results have important implications. To our knowledge this is the first report of restriction endonuclease fragment analysis of any higher plant mtDNA, and we think it demonstrates the

application of a technique which can be used in the analyses of cytoplasmically inherited phenomena. Our results show that a maternally inherited difference in mtDNA is associated with the Texas male-sterile cytoplasm. These observations suggest that the factors conditioning cytoplasmic male sterility and the cytoplasmic inheritance of susceptibility to *B. maydis* and *P. maydis* are located on the mitochondrial genome. Although we cannot disregard chloroplasts or other cytoplasmic DNA's as potential carriers of these traits, the preferential effect of the host-specific fungal toxins on mitochondria from cms T lines (*5*), together with the restriction endonuclease data, constitute strong evidence that the mitochondrion is the organelle involved in the inheritance of the traits.

C. S. LEVINGS, III
Department of Genetics, North Carolina State University, Raleigh 27607
D. R. PRING
Agricultural Research Service, U.S. Department of Agriculture, and *Department of Plant Pathology, University of Florida, Gainesville 32611*

References and Notes

1. M. M. Rhoades, *Science* **73**, 340 (1931).
2. D. N. Duvick, *Adv. Genet.* **13**, 1 (1965).
3. P. H. Harvey, C. S. Levings, III, E. A. Wernsman, *Adv. Agron.* **24**, 1 (1972).
4. G. L. Scheifele and R. R. Nelson, *Plant Dis. Rep.* **53**, 186 (1969); ———, C. Koons, *ibid.*, p. 656; J. E. Ayers, R. R. Nelson, C. Koons, G. L. Scheifele, *ibid.* **54**, 277 (1970); G. L. Scheifele, W. Whitehead, C. Rowe, *ibid.*, p. 501; A. L. Hooker, D. R. Smith, S. M. Lin, J. B. Beckett, *ibid.*, p. 708.
5. R. J. Miller and D. E. Koeppe, *Science* **173**, 67 (1971); J. C. Comstock, C. A. Martinson, B. G. Gengenbach, *Phytopathology* **63**, 1357 (1973).
6. D. Nathans and H. O. Smith, *Annu. Rev. Biochem.* **44**, 273 (1975).
7. R. Kolodner and K. K. Tewari, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 1830 (1972); D. M. Shah and C. S. Levings, III, *Crop Sci.* **14**, 852 (1974); D. Pring, *Plant Physiol.* **53**, 677 (1974).
8. D. Freifelder, *Methods Enzymol.* **21D**, 153 (1971).
9. D. M. Shah and C. S. Levings, III, in preparation.
10. The RI endonuclease from *E. coli* was a gift from M. Edgell, University of North Carolina, Chapel Hill. The RI restriction buffer contained 0.1*M* tris(hydroxymethyl)aminomethane-hydrochloride (tris-HCl), 5 m*M* $MgCl_2$, and 50 m*M* NaCl (*p*H 7.5). Digestions were at 37°C for 1 hour.
11. The dIII endonuclease from *H. influenzae* was purchased from Miles Laboratories, Inc. The dIII restriction buffer contained 6 m*M* tris-HCl, 6 m*M* $MgCl_2$, 50 m*M* NaCl (*p*H 7.5), plus 5 μg of bovine serum albumin per 50 μl of digestion volume. Digestions were at 37°C for 1 hour.
12. The agarose gel electrophoresis and staining procedures were similar to those of M. Thomas and R. W. Davis [*J. Mol. Biol.* **91**, 315 (1975)] except that ethidium bromide was not incorporated into the gels. Gels measured 0.5 to 0.6 cm by 12 to 16 cm; a contant voltage of 1.5 to 1.8 volt/cm was applied.
13. P. A. Sharp, B. Lugden, J. Sambrook, *J. Biochem.* **12**, 3055 (1973).
14. The following maize lines of hybrids in both normal and Texas male-sterile cytoplasm were examined by restriction endonuclease fragment analysis: F44, T204, W64A, B37 × NC236, and NC7 × T204.
15. C. A. Hutchison, III, J. E. Newbold, S. S. Potter, M. H. Edgell, *Nature (London)* **251**, 536 (1974).
16. R. Wells and J. Ingle, *Plant Physiol.* **46**, 178 (1970); C. J. Leaver and M. A. Harmey, *Biochem. Soc. Symp.* **38**, 175 (1973); D. M. Shah and C. S. Levings, III, *Crop Sci.* **14**, 852 (1974).
17. D. M. Shah and C. S. Levings, III, *Crop Sci.* **13**, 709 (1973).
18. Journal series paper No. 4870, North Carolina Agricultural Experiment Station, Raleigh, and cooperative investigation, Agricultural Research Service, U.S. Department of Agriculture, and Institute of Food and Agricultural Sciences, University of Florida, Gainesville; Florida Agricultural Experiment Stations journal series No. 8018. We thank H. E. Warmke and J. R. Edwardson for seed and helpful discussions, and R. G. Hutchins, J. Mooneyhan, and D. M. Shah for technical assistance. Seed of W64A was supplied by Clyde Black and Sons, Ames, Iowa. Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

11 December 1975; revised 26 March 1976

Aggression and Mating Success in Male Spider Mites

Abstract. *Male* Tetranychus urticae *search for and defend quiescent pharate females. Intruding males may be threatened or attacked. Fights involve pushing and grappling with the forelegs, jousting with the mouthparts, and entangling the opponent with silk. In these encounters larger males usually win. Sole possession of a female at her ecdysis virtually ensures successful mating.*

In polygynous mating systems the ability to conquer or intimidate other males is an important component of male fitness (*1*). Despite the relative paucity of analytical studies of invertebrates, it is evident that sexual selection generates aggressive interactions within many groups of arthropods (*2*). Agonistic behavior has been reported in male spider mites (*3, 4*), but its significance has not been recognized. We found that aggressive interactions between competing males of *Tetranychus urticae* Koch are frequent and that they determine the ultimate success or failure in mating.

Spider mites are phytophagous colonizing organisms with a short life cycle (minimum, 10 days) (*5*) and a high intrinsic rate of increase (*6*). In *T. urticae* the tertiary sex ratio is generally 3 : 1 (female to male) (*6*). While males are sexually capable throughout their adult lives, normally only teneral virgins are available as potential mates. As a consequence, the functional sex ratio is skewed toward males. Sperm from the first mating has precedence (*7*); therefore, competition for mates is intense and male aggression is important.

The mites used in our study were from the Ohio State University strain and the Sambucus strain (wild type and albino). The two genotypes of the Sambucus strain are sexually compatible and mating is nonassortative (*8*). Cultures were maintained on kidney bean plants and observations were made on isolates on 12- or 17-mm bean leaf disks pressed onto wet cotton and kept under constant light at 27° ± 2°C.

Female deutonymphs (penultimate instar) spin strands of silk, settle on the leaf surface, and become quiescent during their final moult. Wandering adult males, guided by the silk webbing (*9*) and a sex attractant (*4*), discover the quiescent females. These females become increasingly attractive over time (*8*), and as ecdysis approaches virtually all are attended. Males assume a characteristic guarding position, resting on the dorsum of the female with their forelegs across her body.

Other males, on encountering the pair, often attempt to climb on top of the female. If the first male fails to respond to the intrusion, the two become co-guarders, vying for the preferred position on the female. Usually, however, the resident male does not tolerate the intruder and, if the challenger is persistent, a fight ensues. Agonistic encounters can be characterized, in order of increasing intensity and decreasing frequency, as follows: (i) intruder retreating without aggression; (ii) one-sided aggressive response or threat, usually by the guarding male, ending with retreat of intruder; (iii) moderate to intense aggressive interaction involving both males and ending

Table 1. Size and success of male *Tetranychus urticae* in aggressive encounters.

Character measured	Number of fights in which winner was:			χ^2(1d.f.)*
	Larger	Equal size	Smaller	
Length of tarsus 1	13	2	3	6.25 (P = .012)
Length of tarsus 4	16	1	1	13.24 (P = .0003)
Distance between prodorsals II	11	2	3	4.57 (P = .033)

*Cases of equal size are not considered.