

readily under milder condensation conditions than $\text{HNO}_3 \cdot 3\text{H}_2\text{O}$, and it is therefore a probable component of PSCs.

The HNO_3 vapor pressure in a cloud of $\text{HNO}_3 \cdot 2\text{H}_2\text{O}$ would be supersaturated relative to $\text{HNO}_3 \cdot 3\text{H}_2\text{O}$, but $S_{\text{HNO}_3 \cdot 3\text{H}_2\text{O}}$ does not exceed the value needed for nucleation (~ 8) until ambient temperatures fall close to the ice frost point (where $S_{\text{HNO}_3 \cdot 3\text{H}_2\text{O}} \sim 9$). We infer that nucleation of $\text{HNO}_3 \cdot 3\text{H}_2\text{O}$ may coincide with the formation of ice clouds (type II PSCs). This sequence would be consistent with the idea (23) that falling ice particles could play a key role in denitrification: excess HNO_3 vapor, present in a cloud of metastable $\text{HNO}_3 \cdot 2\text{H}_2\text{O}$, would be available for rapid, efficient transfer to any $\text{HNO}_3 \cdot 3\text{H}_2\text{O}$ on the surface of a falling ice particle.

Temperatures below the ice point are much more prevalent in Antarctica than in the Arctic. The mechanism proposed here would help explain (23, 24) observed denitrification in both polar regions, (7, 8) and may contribute to the associated north-south asymmetry in the severity of O_3 loss. Thus, understanding the complex thermodynamics of hydrates of HNO_3 may be crucial to understanding the mechanisms for severe depletion of polar O_3 .

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- Nitric acid and water vapor concentrations were retrieved with line parameters (positions, strengths, and lower state energies) from the HITRAN database [L. S. Rothman *et al.*, *J. Quant. Spectrosc. Radiat. Transfer* **48**, 469 (1992)]. Nitric acid line strengths were multiplied by 1.14 to obtain agreement with the absolute measurements of R. D. May and C. R. Webster [*ibid.* **38**, 5 (1987)].
- We calibrated the temperature scale of Fig. 3 by fitting the H_2O vapor pressures of ice-containing samples ($\text{HNO}_3 \cdot 3\text{H}_2\text{O}$ and higher hydrate triple points) to known ice vapor pressures (21). This accounted for both the temperature difference of the cold spot over the cold button (see inset in Fig. 1) and the absolute calibration of the resistance temperature device (RTD) temperature sensor. This amounted to a ± 1 K correction of the RTD reading (precision ± 0.2 K) over our experimental temperature range. Statistical deviations in the reproducibility of the H_2O vapor measurement correspond to a ± 0.4 K uncertainty in the relative temperature. Statistical uncertainties (2σ) for $\Delta H^\circ/R$ and $\Delta S^\circ/R$ (where R is the gas constant) are listed in Fig. 3. Uncertainties of $\sim 10\%$ and $\sim 20\%$ in the H_2O and HNO_3 line strengths (17), respectively, are included in the $\Delta S^\circ/R$ uncertainties.
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Identification of a Whitefly Species by Genomic and Behavioral Studies

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An introduced whitefly species, responsible for over a half billion dollars in damage to U.S. agricultural production in 1991, is morphologically indistinguishable from *Bemisia tabaci* (Gennadius). However, with the use of polymerase chain reaction-based DNA differentiation tests, allozymic frequency analyses, crossing experiments, and mating behavior studies, the introduced whitefly is found to be a distinct species. Recognition of this new species, the silverleaf whitefly, is critical in the search for management options.

An introduced whitefly had a large impact on the production of many food and fiber crops in the southern United States in 1991 (1, 2). Integrated crop management and increased applications of currently available pesticides failed to curb the infestations. The resulting agricultural losses have been estimated at over a half billion dollars (2).

Whiteflies of the type referred to as the sweet-potato whitefly, *Bemisia tabaci* (Gennadius) "type A" or "cotton strain," have been present in the United States for approximately 100 years (3) without causing losses of the magnitude experienced in 1991. The newly introduced variant that apparently caused the 1991 damage became known as "superbug" (4) ("type B" or "poinsettia strain") and, based on morphological similarities with the sweet-potato whitefly, has been thought to be a strain of *B. tabaci* (5). Isozyme (5, 6) and biological (7) studies indicate differences between whiteflies of types A and B. A national plan for research on "*B. tabaci*" has been established (8).

The sweet-potato whitefly first was de-

scribed as *Aleyrodes tabaci* and was isolated from tobacco in Greece (9). Since that time, the whitefly has been redescribed in synonymy 22 times and has been recorded from nearly all tropical and subtropical regions (10–12). However, the systematics of this group of whiteflies and of the two whiteflies predominant in the United States must be determined because control strategies based on existing knowledge of "*B. tabaci*" (13) may not be successful when applied to different species.

Species may be defined as "natural populations that are reproductively isolated from one another and that follow distinct and independent evolutionary paths" (14). We used crossing experiments and mating behavior studies to analyze reproductive isolation and genomic studies to elucidate the evolutionary paths followed by the two whitefly types.

All possible crosses among three whitefly populations were performed (Table 1). Two colonies (A1 and A2) were of type A whiteflies, and one colony (B) was of type B whiteflies. To ensure that no mating had occurred prior to placing whiteflies in pairs, leaves with late-instar whitefly nymphs were removed from respective colonies 15 to 18 hours preceding the experiment; *B. tabaci* females are not receptive to males for mating the first 20 hours after emergence

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(15). Newly eclosed, unmated adults were removed from the leaves, their sexes were determined, and they were placed as single pairs in leaf cages. After 5 days of mating and oviposition, each parent was collected and identified with the use of isoelectric focusing electrophoresis (IEF). Nymphal development was observed until the stage preceding adult eclosion, at which time leaves with sessile nymphs were excised

from plants and held in petri dishes containing moist filter paper to allow F1 emergence. As adults emerged, they were collected, their sexes were determined, and they were identified by IEF.

Whiteflies exhibit haplo-diploidy, that is, males (haploid) are produced parthenogenetically from unfertilized eggs and females (diploid) are produced from fertilized eggs (16). Therefore, the production of F1 females indicates transferral of gametes in a cross. F1 females were produced from crosses within each culture, and from crosses between whiteflies from A1 and A2 cultures (even though these cultures had been geographically isolated for over 11 years), but not from crosses between type A and type B whiteflies (Table 1). Thus, there is a failure of gamete transfer between types A and B.

Courtship and mating behavior (15) was studied with a video camera mounted on a $\times 8$ binocular microscope. Five males and five females, all of which had eclosed in the

previous 12 hours, were placed in a Plexiglas observation cell (6 mm in diameter, 1.5 mm deep). Each cell was pressed to the abaxial surface of a lima bean leaf, and behavior was observed through a perforated cover of plastic wrap. Events were recorded on a time-lapse recorder set at 2.4 pictures per second. Three repetitions of the four crosses (A females $\times$ A males, B females $\times$ B males, A females $\times$ B males, and B females $\times$ A males) were conducted for a total of 15 pairs per cross. Courtship behavior (Fig. 1A) was observed and the number of copulation events (Fig. 1B) was recorded for 24 hours. After monitoring, all adults were collected and identified by IEF.

Whiteflies of the same type completed courtship behavior and copulated. The average numbers of copulation events in 24 hours was 5.7 for the A female $\times$ A male cross and 5.0 for the B female $\times$ B male cross. Although whiteflies in the interstrain crosses exhibited courtship behavior, none of the pairs copulated.

Isoelectric focusing (5) was used to identify enzymes, representing 18 loci, for each

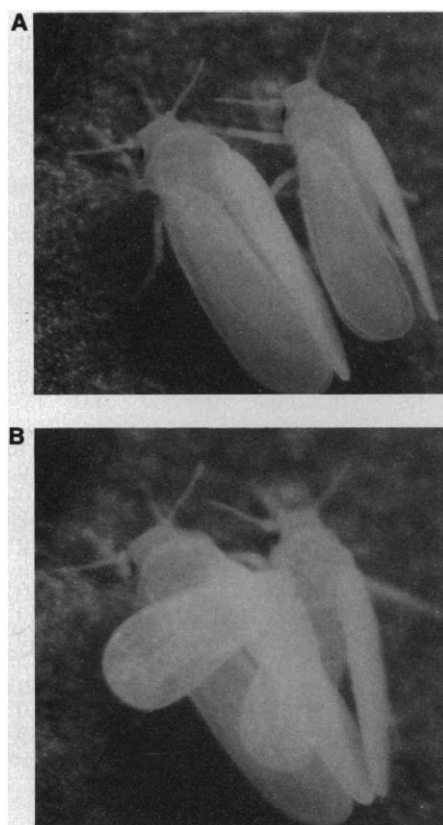


Fig. 1. (A) Male (right) and female (left) whiteflies exhibiting courtship behavior. Individuals will orient to each other in parallel fashion. (B) Whiteflies in copula. Copulation lasts for an average of 134.5 s in the sweet-potato whitefly (type A) and 119.8 s in the silverleaf whitefly (type B).

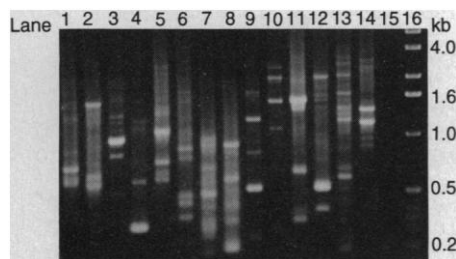


Fig. 2. Single primer PCR amplification of DNA isolated from type A and type B whiteflies. Lanes 1, 3, 5, 7, 9, 11, and 13 contain amplification products derived from type A whiteflies with the primers (GACAC)₃, (GACA)₄, (TCC)₅, (AGG)₅, (ACTG)₄, (CAG)₅, and (GTG)₅, respectively. Lanes 2, 4, 6, 8, 10, 12, and 14 contain amplification products derived from type B whiteflies with the primers (GACAC)₃, (GACA)₄, (TCC)₅, (AGG)₅, (ACTG)₄, (CAG)₅, and (GTG)₅, respectively. Lane 15 represents a control PCR reaction devoid of genomic DNA. Lane 16 contains DNA size markers with some of the sizes indicated in kilobases (kb) on the right.

Table 1. Average number of F1 offspring ($\pm$ standard deviation, SD) produced per mating pair in crossing experiments. A1, colony of type A whiteflies established in 1981, introgressed with field-collected whiteflies until 1987. A2, colony of type A whiteflies established in 1981, maintained without introgression. B, colony of type B whiteflies established in 1991. All colonies originated from whiteflies collected in Imperial County, California. Colonies were maintained on *Phaseolus vulgaris* L. as described (5). Number of crosses, *n*.

Cross (<i>n</i>)	Females (SD)	Males (SD)
A1 female $\times$ A1 male (10)	2.80 (2.60)	6.40 (4.80)
A2 female $\times$ A2 male (9)	3.88 (3.37)	6.00 (4.00)
B female $\times$ B male (8)	6.43 (5.31)	6.43 (6.80)
A1 female $\times$ A2 male (8)	2.13 (3.63)	9.25 (5.07)
A2 female $\times$ A1 male (7)	11.14 (5.11)	9.86 (6.87)
A1 female $\times$ B male (13)	0.00	9.50 (4.67)
B female $\times$ A1 male (14)	0.00	9.71 (8.15)
A2 female $\times$ B male (7)	0.00	19.57 (4.56)
B female $\times$ A2 male (9)	0.00	15.63 (11.18)

Table 2. Origins of type A and type B whiteflies used for genetic distance analysis. Populations of each whitefly type were maintained in culture (type A) or collected from the field (type B). Four type A cultures (TP2, RC1, RC2, and TB) were derived from a parent culture (TP1), which was established in 1981 from whiteflies taken from Imperial County, California, and periodically introgressed with field-collected material until 1987. Another type A culture (JD) was established in 1981 and maintained on sweet potato without introgression. Populations of type B whiteflies were collected from 11 plants. Populations CIT and GRP were collected in Riverside County, California; populations ALF through TAN were collected in Imperial County, California; the PST population was collected in Bakersfield, California; and the TOM population was collected in Bradenton, Florida.

Date	Group name	Rearing host
<i>Type A colonies established</i>		
6/1/1981	TP1	Bean
8/1/1981	JD	Sweet potato
<i>Subcultures from TP1</i>		
11/11/1990	TP2	Bean
6/15/1990	RC1	Bean
6/15/1990	RC2	Bean
12/4/1990	TB	Bean
<i>Type B whiteflies collected</i>		
9/9/1990	CIT	Citrus
9/9/1990	GRP	Grape
9/23/1990	ALF	Alfalfa
9/23/1990	CAN	Cantaloupe
12/13/1990	CAU	Cauliflower
12/13/1990	FIG	Fig
12/13/1990	LAN	Lantana
12/13/1990	LET	Lettuce
12/13/1990	TAN	Tangelo
12/19/1990	PST	Poinsettia
12/30/1990	TOM	Tomato

Table 3. Allelic frequencies of polymorphic loci. For each enzyme, the allelic frequencies in any given population sum to 1. Allele represents the ratio between the distance traveled by the protein, measured from the

cathode, and the distance from the anode to the cathode. Populations (TP1 through TAN) are described in Table 2.

Enzyme	Allele	Type A						Type B										
		TP1	TP2	RC1	RC2	TB	JD	TOM	PST	CIT	GRP	CAN	FIG	LAN	LET	CAU	ALF	TAN
Acid phosphatase (ACP)	0.14	1.0	0.95	0.65	0.75	1.0	0.80	1.0	0.95	0.90	0.85	0.85	1.0	1.0	1.0	1.0	0.95	0.75
	0.20	0.0	0.05	0.35	0.25	0.0	0.20	0.0	0.05	0.10	0.15	0.15	0.0	0.0	0.0	0.0	0.05	0.25
Adenylate kinase II (AK II)	0.62	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.90	0.85	0.95	0.95	1.0	1.0	1.0	0.95	0.90	0.90
	0.64	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.10	0.15	0.05	0.05	0.0	0.0	0.0	0.05	0.10	0.10
Esterase IV (EST IV)	0.42	0.0	0.0	0.0	0.0	0.0	0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.44	1.0	1.0	1.0	1.0	1.0	0.25	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.43	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.0	0.0	0.0
	0.48	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.95	1.0	1.0	1.0
	Null	0.0	0.0	0.0	0.0	0.0	0.50	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fumerase (FUM)	0.18	0.30	0.25	0.30	0.15	0.25	0.20	0.0	0.10	0.50	0.50	0.45	0.50	0.50	0.50	0.50	0.60	0.50
	0.20	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.22	0.50	0.40	0.70	0.35	0.10	0.50	1.0	0.90	0.50	0.50	0.55	0.50	0.50	0.50	0.50	0.40	0.50
	0.24	0.0	0.0	0.0	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.29	0.15	0.10	0.0	0.10	0.20	0.20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.42	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Hexokinase (HK)	0.54	0.15	0.15	0.10	0.45	0.25	0.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.60	0.85	0.85	0.90	0.55	0.75	0.85	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Malate dehydrogenase II (MDH II)	0.20	0.15	0.25	0.20	0.40	0.10	0.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.15	0.15
	0.37	0.0	0.0	0.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.10	0.0
	0.47	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25	0.0	0.0
	0.56	0.85	0.65	0.65	0.60	0.90	0.85	0.85	0.40	0.90	1.0	1.0	0.95	0.95	1.0	0.45	0.65	0.85
	0.64	0.0	0.10	0.0	0.0	0.0	0.0	0.15	0.60	0.10	0.0	0.0	0.05	0.05	0.0	0.30	0.10	0.0
Phosphoglucose isomerase (PGI)	0.16	1.0	1.0	1.0	0.95	1.0	0.85	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.20	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.29	0.0	0.0	0.0	0.05	0.0	0.15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phosphoglucomutase (PGM)	0.17	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
	0.30	1.0	1.0	0.95	1.0	1.0	0.90	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.42	0.0	0.0	0.05	0.0	0.0	0.10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

of 17 whitefly populations (Table 2). Allelic frequencies were used to calculate genetic distances (17).

Allelic polymorphisms were present for 8 of the 14 enzymes evaluated (Table 3). Considerable variation existed among the various populations within each whitefly type. Fixed allelic differences between type A and type B whiteflies were present at three (EST IV, PGI, and PGM) of the eight polymorphic loci. Comparison of the allele frequencies of type A and type B whiteflies resulted in a genetic distance (D) value of 0.24, which is indicative of a species (17).

Among populations within each whitefly type, no fixed allelic differences were observed, yet there were polymorphisms (Table 3). For type A whiteflies, the highest D value (0.04) was obtained from the comparison of the RC2 and JD populations. The highest D values computed from comparisons among the 11 populations of type B whiteflies was 0.01, again demonstrating the high degree of genetic similarity.

Genomic DNA of laboratory-reared type A and type B whiteflies was prepared from 50 to 100 μ l of frozen whiteflies (18–20). The genomic DNA was analyzed with the use of polymerase chain reactions (PCR) (21–23).

A unique set of amplification products was generated for each type of whitefly with each of the seven primers (Fig. 2). The percentage of similarity between the two whitefly types was defined as the percentage of the total number of amplified DNA fragments from both whiteflies that comigrated.

Three populations of each whitefly type [A type: TP1, JD, and TP2; B type: CAU, PST, and TOM (Table 2)] were analyzed by PCR. Analysis of the amplification products generated from each of the primers, (CAG)₅, (TCC)₅, and (GTG)₅, indicated that populations of the same type shared 80 to 100% similarity, but less than 10% similarity was observed between types.

Our work provides evidence that two whiteflies types which, due to morphological similarity, have previously been classi-

fied as strains of a single species are in fact distinct species. Because the type B whitefly has been shown to be correlated with a disorder in squash known as silverleaf (7), we propose that the whitefly previously named type B now be referred to as the silverleaf whitefly.

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 13. The name *B. tabaci* currently is synonymous with 22 names in the Aleyrodidae and has been thought to apply to a widespread and morphologically variable species (10), with substantial morphological plasticity in certain characters within populations [L. A. Mound, *Proc. R. Entomol. Soc. London Ser. A*, **38**, 171 (1963)]. Among these widely dispersed populations is variability in demographic parameters [D. A. Powell and T. S. Bellows, Jr., *Environ. Entomol.* **21**, 359 (1992)]. Although developmental time varies with host plant, development from egg to adult typically is accomplished in 18 to 30 days at 25°C. Adult longevity may span 10 to 22 days, and total oviposition may range from 30 to 300 eggs per female; averages above 200 eggs per female on favorable host plants are common. *B. tabaci* has been reported from 420 plant species in 74 families worldwide (12). Natural enemies of *Bemisia tabaci* include parasitic Hymenoptera (members of the aphelinid genera *Encarsia* and *Eretmocerus*); predaceous Coleoptera, Neuroptera, and Acarina; and parasitic fungi [D. J. Greathead and F. D. Bennett, *Biocontrol News Inf.* **2**, 7 (1981)]. *Bemisia tabaci* transmits 20 viruses [D. N. Byrne, T. S. Bellows, Jr., M. P. Parrella, in *Whiteflies: Their Bionomics, Pest Status and Management*, D. Gerling, Ed. (Intercept, United Kingdom, 1990), p. 227], which cause over 40 diseases of vegetable and fiber crops [J. K. Brown and J. Bird, *Plant Dis.* **76**, 220 (1992)].
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 17. The genetic distance (D) is an estimate of the number of allelic substitutions per locus in two populations. D values for comparisons among a number of vertebrates and invertebrates are 0.00 to 0.05 between populations or races, 0.02 to 0.20 between subspecies, 0.1 to 2.0 between species, and more than 1.0 between genera [M. Nei, in *Population Genetics and Ecology*, S. Karlin and E. Nevo, Eds. (Academic, New York, 1976), p. 723; F. J. Ayala *Population and Evolutionary Genetics: A Primer* (Benjamin-Cummings, Menlo Park, CA, 1985)]. We determined both interstrain (using pooled data from all populations) and intrastrain D values. Populations within each strain showing the most disparate allelic frequencies were compared in order to show the maximum amount of intrastrain genetic differentiation. We also compared two other congeneric but morphologically distinct species, *Trialeurodes vaporariorum* (Westwood) and *Trialeurodes abutilonea* (Haldeman) to determine if the D value between these two species is consistent with the D values recorded for comparisons between other animal species. After examining 16 loci using 14 enzyme stains, we obtained a D value of 0.83, indicative of separate species.
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 20. The original protocol (18) was modified by replacing the final ammonium acetate and isopropanol precipitations of DNA with a precipitation that used 0.1 M NaCl and 2.0 volumes of 95% ethanol (19). In addition, whiteflies were pulverized in microfuge tubes after addition of the lysis buffer.
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 23. Polymerase chain reactions [M. K. Mullis and F. A. Falcon, *Methods Enzymol.* **155**, 335 (1987)] were performed in 20- μ l reactions containing 10 mM tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.1% Triton X-100, 200 μ M each of dATP, dCTP, dGTP, and dTTP (Pharmacia), 0.1 unit of Taq I DNA polymerase (Promega), 2 μ M of a single oligonucleotide primer, and 50 to 200 ng of whitefly genomic DNA (21). Oligonucleotide primers represented repeated DNA sequence motifs as follows: 5'-dCAGCAGCAGCAGCAG-3' (18), 5'-dGACAGACAGACAGACA-3' [J. Epplen, *J. Hered.* **79**, 409 (1989)], 5'-dGACAGCAGACAGCAG-3' (22) [K. Weising *et al.*, *Nucleic Acids Res.* **17**, 10128 (1989)], 5'-dTCCTCCTCCTCCTCC-3' (22), 5'-dACTGACTGACTGACTG-3' (22), 5'-dAGGAGGAGGAGGAGG-3', and 5'-dGTGGTG-GTGGTG-3'. In the text, these primers have been designated (CAG)₅, (GACA)₄, (GACAC)₃, (TCC)₅, (ACTG)₄, (AGG)₅, and (GTG)₅, respectively. Amplification reactions consisted of 30 cycles in an Ericomp Dual Block system following one cycle at 95°C for 5 min. Each cycle consisted of a temperature regime of 95°C for 0.5 min, 60°C or 52°C for 0.5 min [60°C for the (CAG)₅ and (GTG)₅ primers and 52°C for the remaining primers], and 70°C for 1.5 min. Electrophoresis of the amplified products was performed for 1.5 hours at 7.0 V/cm in 1.5% agarose. The oligonucleotide primers used in these studies were synthesized in the bioinstrumentation facility at the University of California, Riverside, and purified by gel filtration with the use of a column of Sephadex G-100 (Sigma) equilibrated with tris-EDTA buffer (19). The DNA from each whitefly type then was amplified using Taq I DNA polymerase and single oligonucleotide primers (21).
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Mediation by G Proteins of Signals That Cause Collapse of Growth Cones

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During development, motion of nerve growth cones ceases on contact with particular targets. The signaling mechanism is unknown. In culture, growth cone collapse can be caused by solubilized embryonic brain membranes, central nervous system myelin, a 35-kilodalton protein isolated from myelin, and mastoparan. Collapse induced by each of these is blocked by pertussis toxin. Thus, collapse of growth cones is mediated by G protein-coupled receptors, which may be activated by proteins associated with the cell surface as well as by soluble ligands.

Inhibitory factors expressed on the surface of cellular targets and along migratory pathways regulate the motion of growth cones (1, 2). These guidance signals help to direct the assembly of neuronal circuitry (1–7) and their presence hinders regeneration of

neuronal processes after brain injury (4). Several inhibitory factors are associated with the cell surface and have been identified in membrane fractions of embryonic brain, optic tectum, somites, and myelin (1, 2, 5–7). Those factors cause collapse of neuronal growth cones in culture (5–7). G proteins, which are known to respond to soluble signals through transmembrane receptors (8), are enriched in the membrane of the growth cone (9). We investigated whether membrane-bound inhibitory ligands function through activation of G proteins.

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