28. E. R. Engdahl, R. D. van der Hilst, R. P. Buland, *Bull. Seismol. Soc. Am.* **88**, 722 (1998).
29. Epicenters are those relocated by Engdahl *et al.* (*28*) and their latest updates. We compared this data set with detailed relocation of a dozen events in the Tonga-Fiji region by Wiens *et al.* (*26*). The difference in epicenters is less than 5 km in most cases and never exceeds 10 km. When available, we used precise focal depths of over 200 events determined by Brudzinski and Chen (*21*, *22*) who modeled the time interval between phases *pP* and *P* using a seismic velocity model specifically developed for this region. On average, focal depths reported by Engdahl *et al.* (*28*) are off by only ±8 km. Thus, the data set used in this study is sufficient to resolve if earthquakes are associated with the active WBZ.
30. B. Isacks, P. Molnar, *Nature* **233**, 1121 (1969).
31. D. Giardini, J. H. Woodhouse, *Nature* **319**, 551 (1986).
32. K. Fischer, K. C. Creager, T. H. Jordan, *J. Geophys. Res.* **96**, 14403 (1991).
33. K. S. Strobach, *Z. Geophys.* **39**, 819 (1973).
34. K. Yamaoka, Y. Fukao, M. Kumazawa, *Rev. Geophys.* **24**, 27 (1986).
35. For this study, we analyzed 50 additional triplicate *P* and *SH* waveforms to extend northward the coverage offered by 270 waveforms in Brudzinski and Chen (*21*, *22*).
36. K. C. Creager, T. H. Jordan, *J. Geophys. Res.* **91**, 3573 (1986).
37. S. Kern, Ed., *P- and S-Wave Velocities in Crustal and Mantle Rocks Under the Simultaneous Action of High Confining Pressure and High Temperature and the Effect of the Rock Microstructure* (Schweizerbart, Stuttgart, Germany, 1982).
38. The latter could be either hydrous minerals or free fluids, such as water or even partial melt (*21*, *22*).
39. U. H. Faul, D. R. Toomey, H. S. Waff, *Geophys. Res. Lett.* **21**, 29 (1994).
40. H. Fujisawa, *J. Geophys. Res.* **103**, 9591 (1998).
41. H. W. Green, P. C. Burnley, *Nature* **341**, 733 (1989).
42. H. W. Green, H. Houston, *Annu. Rev. Earth Planet. Sci.* **23**, 169 (1995).
43. T. Inoue, D. J. Weidner, P. A. Northrup, J. B. Parise, *Earth Planet. Sci. Lett.* **160**, 107 (1998).
44. T. Katsura, E. Ito, *J. Geophys. Res.* **94**, 15663 (1989).
45. S. H. Kirby, W. B. Durham, L. A. Stern, *Science* **252**, 216 (1991).
46. C. Meade, R. Jeanloz, *Science* **252**, 68 (1991).
47. P. G. Silver *et al.*, *Science* **268**, 69 (1995).
48. The configuration of the slab remnant shown in Fig. 3 includes the group of isolated deep earthquakes near 15°S and 170°E. Despite a large aseismic region between 15° and 18°S, the distribution of hypocenters and fault plane solutions of the isolated earthquakes is similar to those illustrated in Fig. 2C (*19*, *20*, *61*). At the present, however, it is unknown if a petrologic anomaly accompanies this cluster of earthquakes, or if this cluster is attached to other outboard earthquakes.
49. M. Barazangi, B. L. Isacks, J. Oliver, J. Dubois, G. Pascal, *Nature* **242**, 98 (1973).
50. C. R. Bina, *Geophys. Res. Lett.* **23**, 3563 (1996).
51. Only the portion of slab below the 410-km discontinuity becomes buoyant because of metastable α-phase (50, 62). However, this portion will never float above the 410-km discontinuity where warmer olivine of the ambient mantle is also in α-phase.
52. A. E. Ringwood, T. Irifune, *Nature* **331**, 131 (1988).
53. Y. Fukao, M. Obayashi, H. Inoue, M. Nenbai, *J. Geophys. Res.* **97**, 4809 (1992).
54. P. M. Shearer, T. G. Masters, *Nature* **355**, 791 (1992).
55. R. D. van der Hilst, E. R. Engdahl, W. Spakman, *Geophys. J. Int.* **115**, 264 (1993).
56. C. Y. Chen, F. A. Frey, *Nature* **302**, 785 (1983).
57. L. H. Kellogg, B. H. Hager, R. D. van der Hilst, *Science* **283**, 1881 (1999).
58. D. L. Turcotte, D. Paul, W. M. White, *J. Geophys. Res.* **106**, 4265 (2001).
59. R. D. van der Hilst, H. Kárason, *Science* **283**, 1885 (1999).
60. Slab material would be difficult to detect with routine seismological experiments when thermal and petrologic effects on seismic wave speeds counteract each other.
61. S. H. Kirby, S. Stein, E. A. Okal, D. C. Rubie, *Rev. Geophys.* **34**, 261 (1996).
62. F. C. Marton, C. R. Blina, S. Stein, D. C. Rubie, *Geophys. Res. Lett.* **26**, 119 (1999).

63. H. Green and an anonymous reviewer provided helpful reviews. Supported by the NSF.

17 January 2001; accepted 21 May 2001

A Mite Species That Consists Entirely of Haploid Females

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The dominance of the diploid state in higher organisms, with haploidy generally confined to the gametic phase, has led to the perception that diploidy is favored by selection. This view is highlighted by the fact that no known female organism within the Metazoa exists exclusively (or even for a prolonged period) in a haploid state. We used fluorescence microscopy and variation at nine microsatellite loci to show that the false spider mite, *Brevipalpus phoenicis*, consists of haploid female parthenogens. We show that this reproductive anomaly is caused by infection by an undescribed endosymbiotic bacterium, which results in feminization of haploid genetic males.

It is commonly thought that no female organism within the Metazoa exists exclusively in a haploid state, because selection favors diploidy in higher organisms (*1*, *2*). There are several theories about the evolution of diploidy as the dominant state, and deleterious mutations (germline and/or somatic) are considered to be the driving force (*3*, *4*). Yet experimental evidence is lacking, and currently no studies on life cycle evolution have been conducted in a higher organism.

Brevipalpus phoenicis Geijskes (Acari: Tenuipalpidae) is a minute phytophagous mite found throughout tropical and subtropical regions. It is polyphagous and is a major pest of many economically important crops such as citrus, coffee, tea, papaya, passion fruit, and palms (*5*). In citrus, it acts as a vector for citrus leprosis virus (Rhabdoviridae), a disease that causes millions of dollars of damage to the Brazilian citrus industry each year (*6*). *Brevipalpus phoenicis*, along with two closely related species, *B. obovatus* and *B. californicus*, is known to reproduce by thelytokous (obligate) parthenogenesis (*7*). Rare males are found in field populations; however, their function is not known (*8*). The closest sexual relative, *B. russulus*, is haplo-diploid, in which unfertilized eggs develop into haploid males (two chromosomes) whereas fertilized eggs develop into diploid females (four chromosomes) (*9*). Haplo-diploidy is characteristic of their superfamily, the Tetranychoidea. All three parthenogenetic species have only two chromosomes in somatic cells (Fig. 1A) (*10*); however, owing to the small size of these chromosomes and their apparent lack of any distinguishable morphological character, it is not known whether this represents the haploid or diploid state.

Although it has been proposed that *B. obovatus* is a haploid parthenogen (*7*, *9*), convincing cytological evidence has been lacking (*11*, *12*). Cytological techniques using fluorescence microscopy provide an accurate way to determine whether these three species are haploid or diploid parthenogens. We used two such techniques, as well as genetic variation at nine microsatellite loci, to show that *B. phoenicis* is indeed a haploid female parthenogen.

Brevipalpus phoenicis females collected from a coffee plantation at the University of Sao Paulo, Piracicaba, Sao Paulo, Brazil, were used to initiate five isofemale lines (lines started with a single immature female) from which eggs were used for the following experiments. Using a fluorescent dye (YOYO-1, Molecular Probes) that stains both DNA and RNA, we visualized the nuclear organizing region (NOR), which is present during early prophase in mitotic divisions in eggs of *B. phoenicis* (*13*, *14*). Homologous pairs of chromosomes will have a NOR or NORs at exactly the same position. We found one NOR to be present during early prophase mitotic divisions in 2-day-old eggs (Fig. 1, B and C) from *B. phoenicis*. The NOR was found at the tip of one chromosome, with no corresponding NOR being present on the second chromosome.

To complement the finding of a single NOR, we also used fluorescent in situ hybridization (FISH) to locate the ribosomal DNA

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(rDNA) region(s) within metaphase chromosomes. Based on the finding of a single NOR, only one region of rDNA should be present. A probe was made from the small subunit 18*S* rDNA of *B. phoenicis* (*15*). The same FISH procedure was followed as that outlined in (*16*), with a slight modification (*17*). The 18*S* rDNA probe from *B. phoenicis* hybridizes at the tip of both sister chromatids of one chromosome, in a metaphase mitotic division (Fig. 1, D and E). No hybridization occurred on the second chromosome. This concurs with the single NOR found with YOYO-1 (Fig. 1, B and C).

We also screened 45 clonal lines of *B. phoenicis* collected from Sao Paulo and Minas Gerais, Brazil, for variation at seven polymorphic microsatellite loci (*18*) and screened two of these clonal lines at two additional loci (Table 1). Ten individuals per clonal line were genotyped, with no differences found within a clonal line. No heterozygous individuals (in a total of 450 individuals), at any locus, were found. These data, along with the finding of a single NOR and only one rDNA region, clearly show *B. phoenicis* to be a haploid female parthenogen.

During the above cytological examination of mitotic chromosomes in eggs, we found large numbers of an endosymbiotic bacterium (Fig. 2) and attempted to discover whether it was involved in female haploid parthenogenesis.

We amplified and sequenced 1487 base pairs (bp) of the bacterial 16*S* rDNA from the isofemale lines used in the cytological experiments using general eubacterial primers (*19*). Only one bacterial 16*S* rDNA sequence was found. When compared with all known bacterial 16*S* rDNA sequences in GenBank, this bacterium's closest relative was an undescribed endosymbiotic bacterium found in the tick *Ixodes scapularis* (*20*) (98% sequence homology), belonging to the phylum *Cytophaga-Flavobacterium-Bacteroides*. To test whether this bacterium is involved in causing parthenogenesis, we treated young adult females from one isofemale line of *B. phoenicis* with the antibiotic tetracycline hydrochloride (*21*). After 3 days of tetracycline treatment, females were allowed to lay eggs for 10 days. The resulting progeny were then scored at the adult stage for sex (Table 2). Young adult females from the same isofemale line were also allowed to lay eggs for 10 days without tetracycline treatment to act as a control. Significantly more males were produced after tetracycline treatment [one-way analysis of variance (ANOVA) after arcsine transformation, $F_{1,21} = 18.69$, $P < 0.001$], with 51% of the progeny being male. This is expected under haplo-diploidy (which is likely to be the ancestral state in *B. phoenicis*), because the females are unfertilized, and curing of a bacterial infection that causes parthenogenesis will result in haploid males. The females that were produced would still be expected to carry the bacterial infection, because tetracycline curing of adults is not 100% effective. More males were produced in the control treatment (~5%) than expected (*10*), and this may be due to the temperature at which the

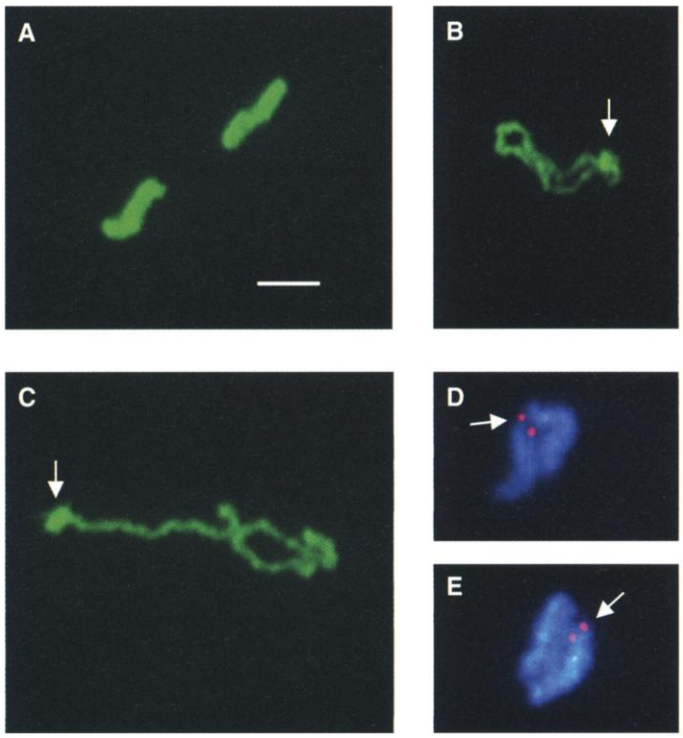

Fig. 1. Chromosomes of *B. phoenicis* obtained from 2-day-old eggs and viewed under a fluorescence microscope. (**A**) Metaphase chromosomes stained with YOYO-1, showing the presence of two chromosomes in a mitotic division. (**B** and **C**) Prophase chromosomes stained with YOYO-1. Arrows indicate a single NOR. (**D** and **E**) FISH experiments. DAPI counterstained metaphase chromosomes are shown after hybridization to the biotinylated 18*S* rDNA probe detected with Cy3-conjugated streptavidin (Jackson ImmunoResearch Labs, West Grove, Pennsylvania). Arrows indicate the hybridization signal (red) on each sister chromatid. There is no corresponding hybridization signal on the second chromosome. Scale bar in (A) indicates 2 μm in (A) through (E).

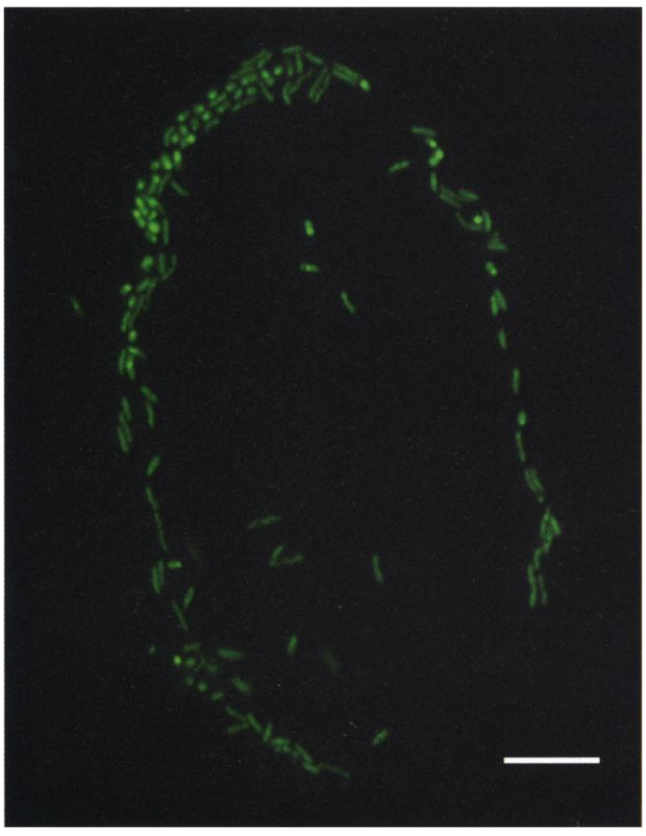

Fig. 2. Bacteria stained with YOYO-1 from a single 2-day-old egg of *B. phoenicis* viewed under a fluorescence microscope. Scale bar, 5 μm.

experiment took place (30°C). In some parthenogenetic *Trichogramma* species infected with the endosymbiotic bacterium *Wolbachia* (which causes the parthenogenesis), rearing at high temperatures (>28°C) can kill the bacteria, resulting in significantly greater male production (*22*).

Using polymerase chain reaction (PCR) primers specific for the bacterium (*23*), all females tested, regardless of the treatment (50 from controls and 80 from tetracycline treatment) were infected with the bacterium, whereas all males tested (17 from controls and 72 from tetracycline treatment) were not infected.

Our results show that females are haploid and that the female haploid parthenogenesis is caused by an endosymbiotic bacterial infection, which results in feminization of genetic males. Once cured of the infection, an adult haploid female will lay haploid male offspring. How the bacterium induces feminization of genetic males is not known. This is the first time feminization by an extrachromosomal factor has been found outside of heterogametic reproductive systems. In the isopod *Armadillidium vulgare*, the bacterium *Wolbachia* also induces feminization (*24*) by blocking the formation of the androgenic gland, which produces the androgenic hormone responsible for male differentiation (*25*). A similar mechanism may occur here, resulting in haploid females instead of haploid males.

The data presented here illustrate that feminization can involve bacteria other than *Wolbachia*. The endosymbiotic bacterium *Wolbachia* has been known to induce a number of reproductive phenotypes, including cytoplasmic incompatibility, parthenogenesis, feminization, and male-killing in various arthropod species (*22*) and has been implicated in sex determination (*26*) and speciation (*27*). We have found another bacterium, unrelated to *Wolbachia*, that causes feminization. Recently, a similar bacterium (with 96% sequence homology for 16*S* rDNA) has been found to be associated with parthenogenesis in several species of parasitic wasps (*28*). This suggests that we should not be looking specifically for *Wolbachia* to explain reproductive abnormalities, as some have done previously (*29–32*).

There remains a possibility that *B. phoenicis* is diploid and that one NOR (and corresponding rDNA) has been lost, as has been found with some thelytokous aphid species (*33*). However, the evidence does not support this. First, the only known cytogenetic mechanisms of parthenogenesis that could result in complete homozygosity at all microsatellite loci studied is gamete duplication or terminal fusion (with no crossing over) (*34*). A single NOR from a homologous pair cannot be lost through gamete duplication or terminal fusion, because loss would in the subsequent generation result in either no NOR at all (which presumably would be lethal) or the homologous pair would be restored (*34*). Second, if curing of the bacterium results in male progeny, then males must be haploid with $n = 1$. Yet the number of chromosomes in male sperm and developing embryos is two (*8*, *9*).

Finally, because *B. phoenicis* appears to live entirely in a haploid state, there is a unique opportunity to study life cycle evolution and the advantages and disadvantages associated with haploidy in a higher organism, where diploidy dominates. Typically, these questions have been studied in bacteria, fungi, moss, algae, and ferns (*1*) where life cycles and ploidy levels are not always dominated by a diploid phase, and therefore their relevance may be questioned. If diploid sexual females of *B. phoenicis* exist or can be generated, or if generation of haploid female *B. russulus* is possible through the *B. phoenicis* bacterium, it may be possible to gain the first empirical data on the relative importance of somatic and germline deleterious mutations for the maintenance of diploidy in animals.

Table 1. Characteristics of nine polymorphic microsatellite loci (for $n = 45$ clonal lines, except loci Brev08 and 09, where $n = 2$ clonal lines) in *B. phoenicis* collected from two different host plant species (citrus and coffee) in Sao Paulo, Brazil. Ten individuals were assayed per clonal line, with no differences being found within a clonal line for each locus. No heterozygous loci were found in a total of 450 individuals. Microsatellites and their flanking sequences can be found in GenBank under accession numbers AF335574 through AF335582.

Locus	Repeat array	*n*	No. of alleles	Frequency of common allele	Frequency of heterozygotes
Brev01	TG	45	3	0.77	0
Brev02	AC	45	5	0.39	0
Brev03	TG	45	4	0.75	0
Brev04	TG	45	3	0.41	0
Brev05	AC	45	4	0.48	0
Brev06	AC	45	4	0.68	0
Brev07	GA_xGA	45	4	0.46	0
Brev08	TG	2	2	0.5	0
Brev09	AC	2	2	0.5	0

Table 2. Sex ratio produced by *B. phoenicis* females treated with 0.2% tetracycline hydrochloride for 72 hours and left to lay eggs for 10 days.

Treatment	No. of females per replicate	No. of replicates	Total no. of female progeny	Total no. of male progeny	Proportion of male progeny
Antibiotic	5	13	86	88	0.506*
Control	5	10	288	17	0.056

*$P < 0.001$ (one-way ANOVA after arcsine transformation).

References and Notes

1. B. K. Mable, S. P. Otto, *BioEssays* **20**, 453 (1998).
2. D. M. Welch, M. Meselson, *Science* **288**, 1211 (2000).
3. A. S. Kondrashov, J. F. Crow, *Nature* **351**, 314 (1991).
4. H. A. Orr, *Genetics* **139**, 1441 (1995).
5. J. S. Kennedy, G. van Impe, T. Hance, P. Lebrun, *J. Appl. Entomol.* **120**, 493 (1996).
6. A. R. Weeks, T. van Opijnen, J. A. J. Breeuwer, *Exp. Appl. Acarol.* **24**, 775 (2000).
7. W. Helle, H. R. Bolland, W. R. B. Heitmans, *Genetica* **54**, 45 (1980).
8. L. P. Pijnacker, M. A. Ferwerda, W. Helle, *Acarologia* **22**, 157 (1981).
9. L. P. Pijnacker, M. A. Ferwerda, H. R. Bolland, W. Helle, *Genetica* **51**, 211 (1980).
10. W. Helle, H. R. Bolland, J. Gutierrez, *Experientia* **28**, 707. (1972).
11. R. A. Norton, J. B. Kethley, D. E. Johnston, B. M. O'Connor, in *Evolution and Diversity of Sex Ratio in Mites and Insects*, D. L. Wrensch, M. A. Ebbert, Eds. (Chapman & Hall, New York, 1993), pp. 8–99.
12. D. L. Wrensch, J. B. Kethley, R. A. Norton, in *Mites: Ecological and Evolutionary Analyses of Life-History Patterns*, M. A. Houck, Ed. (Chapman & Hall, New York, 1994), pp. 282–343.
13. We screened eggs from 1 day old up to 10 days old to determine the best egg age for prophase and metaphase plates. At all egg ages, cells contained only two chromosomes, with the best prophase and metaphase plates found with 2-day-old eggs (past 256 nuclei stage). Thus, 2-day-old eggs of *B. phoenicis* were fixed in a solution of 96% ethanol:chloroform:acetic acid (ratio, 6:3:1) for 10 min. Fixed eggs were then transferred to a slide pretreated in acid ethanol (1% HCl in 100% ethanol) and dissected in 60% acetic acid. Slides were then placed on a heating block at 45°C until the acetic acid had almost evaporated (causing the heat fixation of chromosomes). The slide was then passed through a series of ethanol dilutions to dehydrate the slides (through 70, 80, and 96% ethanol solutions for 30 s per solution). Slides were then air-dried and stored at –20°C until used for either YOYO-1 staining or FISH.
14. Fixed slides were taken from storage at –20°C and again passed through the same ethanol series as above and air-dried. They were then soaked in phosphate buffer solution (PBS) (pH 7.3) (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, and 1.4 mM KH_2PO_4) containing 1% Triton X-100 (Sigma) for 5 min. Subsequently, 50 μl of YOYO-1 (Molecular Probes), diluted in PBS buffer to 500 nmol/ml, was added to the slide, covered with a coverslip, and incubated in the dark for 20 min at room temperature. The coverslip was removed under running water, the slide was washed for 30 s, and 20 μl of DABCO antifade [0.233 g of DABCO (Sigma), 800 μl of sterile

H_2O, 200 μl of 1 M tris-HCl (pH 8.0), and 9 ml of glycerol] was then added to the slide. A coverslip was placed on the slide and sealed with nail varnish. Slides were stored at 4°C until viewed under a fluorescence microscope.
15. The probe was made from the 18S ribosomal DNA of *B. phoenicis*. General arthropod primers from C. Simon *et al.* [*Ann. Entomol. Soc. Am.* **87**, 651 (1994)] were used to amplify the 18S rDNA from a mixture of DNA from the five isofemale lines of *B. phoenicis*. Once amplified, the product was cloned into a pGEM-T vector (Promega) and sequenced to determine whether it was really mite 18S rDNA. The insert was then cut out of the vector using restriction enzymes, cleaned, and labeled with biotin by nick-translation using a BioNick Labeling System (Gibco-BRL, Life Technologies).
16. K. Sahara, F. Marec, W. Traut, *Chromosome Res.* **7**, 449 (1999).
17. Chromosome preparations were digested with RNase A [100 μg/ml in 2× saline sodium citrate (SSC) buffer; 1× SSC is 0.15 M sodium citrate and 0.015 M NaCl (pH 7.0)] for 1 hour at 37°C and washed two times for 15 min in 2× SSC. After FISH, chromosomes were observed in a Zeiss Axioplan 2 microscope. Black and white images were recorded with a cooled charge-coupled device camera MEGA F-View II (Soft Imaging System, Münster, Germany) and then pseudocoloured [blue for 4′,6′-diamidino-2-phenylindole dihydrochloride (DAPI) and red for Cy3] and superimposed with the aid of an image-processing program (Adobe Photoshop Version 5.0).
18. Two hundred immature *B. phoenicis* females were collected from coffee and citrus plantations in Sao Paulo and Minas Gerais, Brazil (two coffee sites and five citrus sites). These were reared in the lab as isofemale lines and screened for genetic variation using the amplified fragment length polymorphism method (AFLP) as mentioned in (*6*). Forty-five genotypically distinct lines were then screened for seven polymorphic microsatellite loci, and two of these lines were screened for two additional loci. Microsatellite loci were isolated using a method developed by A. R. Weeks and J. A. J. Breeuwer. For information regarding this technique, please e-mail the corresponding author.
19. For identifying the bacteria, we used general eubacterial primers to amplify the 16S rDNA (fD2 and rP2) from W. G. Weisburg *et al.* [*J. Bacteriol.* **173**, 697 (1991)]. PCR products from each of the five isofemale lines were then cloned into a pGEM-T vector. Owing to the nature of the primers, it is possible that nonendosymbiotic bacteria could be amplified and then wrongly classified as the endosymbiotic bacteria we found through cytology. To control for this, we extracted vectors from five recombinant colonies from each of the five isofemale lines and sequenced all inserts. All 25 inserts contained identical copies of the same sequence (GenBank accession number AF350221).
20. T. J. Kurtti, U. G. Munderloh, T. G. Andreadis, L. A. Magnarelli, *J. Invertebr. Pathol.* **67**, 318 (1996).
21. Two hundred adult female *B. phoenicis* were collected from an egg wave (500 female mites from one of the five isofemale lines were allowed to lay eggs for 2 days, then removed) 2 days after their final molt. One hundred adults were then treated with tetracycline. Leaf discs (1 cm in diameter) of the common bean, *Phaseolus vulgaris*, were floated in a solution of 0.2% tetracycline hydrochloride and distilled water. Five adult mites per leaf disc were left to feed for 72 hours at constant temperature (30°C). At the same time, 60 adult mites were set up in exactly the same way, except that no tetracycline was added to the distilled water, to act as controls. After 72 hours, the tetracycline-treated mites as well as the control mites were transferred to a leaf of *P. vulgaris*, and left to recover for 24 hours at 30°C. Surviving mites from each treatment were then set up as follows: five mites per leaf disc (1 cm in diameter) floated on cotton wool soaked in water at 30°C. Mites were left to lay eggs for 10 days, at which time adults were taken from the leaf disc, leaving the leaf discs and developing eggs at 30°C. Upon reaching the adult stage (2 weeks), the sex of the progeny was scored.
22. R. Stouthamer, J. A. J. Breeuwer, G. D. D. Hurst, *Annu. Rev. Microbiol.* **53**, 71 (1999).
23. Primers were designed based on the sequence in (*19*) and other known bacterial 16S rDNA sequences to specifically amplify only this bacterium. The PCR primers were: 5′-CCCATACAATCTTGCTAGCG (forward) and 5′-CTCAAGGTGTCAAAATACATTC (reverse). These primers amplify a fragment 832 bp in length.
24. F. Rousset, D. Bouchon, B. Pintureau, P. Juchault, M. Solignac, *Proc. R. Soc. London Ser. B* **250**, 91 (1992).
25. G. Martin, P. Juchault, O. Sorokine, A. van Dorsselaer, *Gen. Comp. Endocrinol.* **80**, 349 (1990).
26. T. Rigaud, P. Juchault, J. P. Mocquard, *BioEssays* **19**, 409 (1997).
27. S. R. Bordenstein, F. P. O'Hara, J. H. Werren, *Nature* **409**, 707 (2001).
28. E. Zchori-Fein, Y. Gottlieb, M. S. Hunter, personal communication.
29. M. J. Perrot-Minnot, R. A. Norton, *Can. Entomol.* **129**, 691 (1997).
30. T. Wenseleers, *et al.*, *Proc. R. Soc. London Ser. B* **264**, 1447 (1998).
31. T. Wenseleers, J. Billen, *J. Evol. Biol.* **13**, 277 (2000).
32. D. A. Grasso, T. Wenseleers, A. Mori, F. Le Moli, J. Billen, *Ethol. Ecol. Evol.* **12**, 309 (2000).
33. R. L. Blackman, J. M. Spence, *Chromosome Res.* **4**, 314 (1996).
34. E. Suomalainen, A. Saura, J. Lokki, *Cytology and Evolution in Parthenogenesis* (CRC Press, Boca Raton, FL, 1987).
35. Supported by a grant from the Netherlands Foundation for the Advancement of Tropical Research (WOTRO). F.M. was supported by a grant (206/00/0750) from the Grant Agency of the Czech Republic (Prague). We thank M. Sabelis for initiation of this project and insightful discussions and comments; C. Omoto for help in field collections; A. Hoffmann, S. Menken, C. Sgro, T. van Opijnen, and A. Janssen for critical comments; and I. Sauman for methodical suggestions.

5 March 2001; accepted 9 May 2001

Gene Families from the *Arabidopsis thaliana* Pollen Coat Proteome

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The pollen extracellular matrix contains proteins mediating species specificity and components needed for efficient pollination. We identified all proteins >10 kilodaltons in the *Arabidopsis* pollen coating and showed that most of the corresponding genes reside in two genomic clusters. One cluster encodes six lipases, whereas the other contains six lipid-binding oleosin genes, including *GRP17*, a gene that promotes efficient pollination. Individual oleosins exhibit extensive divergence between ecotypes, but the entire cluster remains intact. Analysis of the syntenic region in *Brassica oleracea* revealed even greater divergence, but a similar clustering of the genes. Such allelic flexibility may promote speciation in plants.

Because self-recognition systems must adapt to the evolution of target molecules, they include some of the most rapidly changing proteins known. Unusual levels of genetic divergence are seen in mate recognition in plants, algae, abalone, and primates (*1–4*); immune responses in animals (*5*); and pathogen defense in plants (*6*). Rapid divergence of molecules controlling mate recognition in flowering plants is essential, considering diversification of most angiosperms occurred only 90 to 130 million years ago. Here, we describe the protein components of the *A. thaliana* pollen coat and show they display remarkable variability.

The complex extracellular pollen coating of many flowering plants uses proteins and lipids to interact selectively with receptive female stigma cells (*7–10*). This coating facilitates communication in plants with dry stigmas, providing a function similar to the lipid-rich exudate on the surface of wet stigmas (*8*). Identification and characterization of the most abundant *Arabidopsis* pollen coat

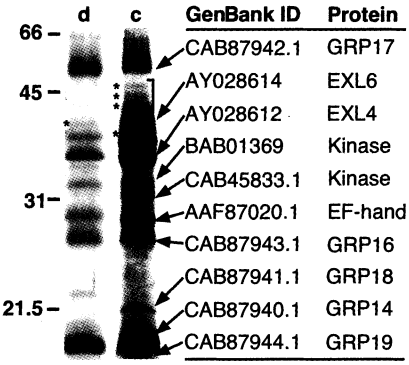

Fig. 1. Purified pollen coat proteins and their identity. Coomassie stained SDS-PAGE with corresponding GenBank ID. Asterisk, EXL6 protein; d, dilute protein sample; c, concentrated protein sample.

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