hours at 32°C (Fig. 4B; note the appearance of VSV-G staining at the cell surface after 60 min only in control cells). Therefore, DAG was required for transport of proteins from the TGN to the cell surface.

Our findings reveal that DAG is required for the recruitment of PKD to the TGN and in the stages leading to the formation of transport carriers in mammalian cells. The obvious challenge now is to determine how DAG is generated in the TGN and how its levels are regulated during protein transport specifically from the TGN to the plasma membrane.

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Identification of a Major Gene Regulating Complex Social Behavior

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Colony queen number, a major feature of social organization in fire ants, is associated with worker genotypes at the gene *Gp-9*. We sequenced *Gp-9* and found that it encodes a pheromone-binding protein, a crucial molecular component in chemical recognition of conspecifics. This suggests that differences in worker *Gp-9* genotypes between social forms may cause differences in workers' abilities to recognize queens and regulate their numbers. Analyses of sequence evolution indicate that regulation of social organization by *Gp-9* is conserved in South American fire ant species exhibiting social polymorphism and suggest that positive selection has driven the divergence between the alleles associated with alternate social organizations. This study demonstrates that single genes of major effect can underlie the expression of complex behaviors important in social evolution.

The evolution of complex social behavior is among the most important events in the history of life (*1*). Interest in the genes underlying the expression of key social traits is strong because knowledge of the genetic architecture will lead to increasingly realistic models of social evolution, while identification of the products of major genes can elucidate the molecular bases of social behavior (*2*). Few studies have succeeded in showing that complex social behaviors have a heritable basis, and fewer still have suggested that variation in these behaviors is attributable to the action of one or few genes of major effect (*3*, *4*). No candidate genes with major effects on key social polymorphisms have been identified previously.

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The fire ant *Solenopsis invicta* displays a fundamental social polymorphism that appears to be under simple genetic control (*5*, *6*). A basic feature of colony social organization, the number of egg-laying queens, is associated with variation at the gene *Gp-9*. In the United States, where this species has been introduced, colonies composed of workers bearing only the *B* allele at *Gp-9* invariably have a single queen (monogyne social form), whereas colonies with workers bearing the alternate, *b* allele have multiple queens (polygyne form) (*4*). The two social forms differ in many key reproductive and life history characteristics (*7*), so that the presence of the *b* allele in a colony of workers with the *b* allele induces a fundamental and far-reaching shift in the social system of this ant. Variation at *Gp-9* has been assessed by starch-gel protein electrophoresis (SGPE) coupled with nonspecific protein staining; thus, the gene product and the mechanisms by which it may influence social behavior were unknown.

We determined the amino acid sequences of several peptide fragments of the GP-9 protein by Edman degradation (*8*). Degenerate deoxyinosine-oligonucleotide primers (*9*) corresponding to the NH_2-terminus and an internal peptide fragment were used to amplify the cDNA recovered from reverse transcription of mRNA (*10*). The amplified fragments were cloned and sequenced. The partial nucleotide sequences of the transcripts then were used to design nondegenerate primers for recovering the full-length mRNA transcripts with a 5′ and 3′ rapid amplification of cDNA ends approach (5′ and 3′ RACE) (*11*). The cap site of *Gp-9* was identified, and its full-length cDNA was found to be 672 base pairs (bp) in length, excluding the polyadenylate tail. The transcript contains an open reading frame of 459 bp, encoding a precursor protein of 153 amino acids (Fig. 1). The mature GP-9 protein, when cleaved of its 19-residue signal peptide (*12*), has an estimated molecular mass of 14.7 kD. Amplification and sequencing of genomic DNA revealed that the *Gp-9* gene is 1700 bp in length, containing five exons and four introns (Fig. 1A).

GenBank BLASTX searches revealed that *Gp-9* most closely resembles genes encoding moth pheromone-binding proteins (PBPs). Although the amino acid sequence identity is modest (26%), PBPs from different moth species generally have low identity (*13*), and the size and structure of GP-9 coincide with the consensus characteristics of proteins of this class. Importantly, GP-9 shares with all other PBPs six characteristically spaced cysteine residues (Fig. 1, B and C) (*14*). Insect PBPs are crucial molecular components in the process of chemical recognition of conspecifics, acting to transport odorant molecules from cuticular pores to receptors on sensory neurons in chemosensilla (*15*). *So-*

lenopsis invicta workers regulate the number and identity of egg-laying queens in a colony by accepting queens that produce appropriate chemical signals and destroying those that do not (*4*, *16*); thus, the core feature of colony social organization, the number of egg-laying queens, is mediated by worker recognition of and subsequent discrimination among queens. The presumed role of GP-9 in chemoreception suggests that the essential distinction in colony queen number between the monogyne and polygyne forms may stem from differences in workers' abilities to recognize queens, differences that are, in turn, associated with the characteristic worker *Gp-9* genotype compositions distinguishing the forms.

Sequence analysis of mRNA transcripts from five queens of each social form collected in northern Georgia, United States, revealed the presence of two distinct transcripts corresponding to the two alleles detected by SGPE (*17*). Both transcripts were isolated from all polygyne queens, which were typed by SGPE as $Gp\text{-}9^{Bb}$ heterozygotes (*18*), whereas only one of the transcripts occurred in monogyne queens, which were typed as $Gp\text{-}9^{BB}$ homozygotes. The association of each transcript sequence with each SGPE-determined *Gp-9* allele was confirmed by identifying the charge-changing amino acid substitution responsible for the different electrophoretic mobilities of the allelic proteins: The acidic glutamic acid residue at position 151 in the *B* allele product is replaced by a basic lysine residue in the *b* allele product (Fig. 1C), causing a decreased net negative charge in the latter protein and its observed lower mobility. The two *Gp-9* alleles differ by nine nucleotide substitutions in the coding regions, all of which are associated with the eight amino acid differences (one synonymous substitution occurs in the codon of a nonconserved residue) (Fig. 1C). This apparent high ratio of nonsynonymous to synonymous substitutions suggests that positive selection has driven the divergence of these alleles (see formal analyses below), consistent with behavioral studies implicating strong diversifying selection on *Gp-9* in the alternate social forms (*5*, *7*, *16*).

We assessed the diversity of *Gp-9* alleles in the introduced range of *S. invicta* in the United States by sequencing the gene in 16 individuals from four localities (California, Texas, Georgia, and Florida). Two individuals of each social form were analyzed from each locality (*19*). The sequenced fragments were amplified from genomic DNA (*20*) and contained the complete gene plus the 3′ flanking region, encompassing 2200 bp. We confirmed the existence of the same two coding region variants detected from the mRNA transcripts, and again these corresponded perfectly with the SGPE-determined *B* and *b* alleles. All polygyne queens had both alleles (*18*), whereas all monogyne queens had only the *B* allele, confirming the link between nucleotide-sequence genotype, SGPE genotype, and social form throughout the introduced range. Little additional nucleotide variation was detected over the 1740 bp of noncoding sequence (*21*).

Previous protein electrophoretic studies of *S. invicta* from the native range in Argentina suggest a more complex relationship between *Gp-9* genotype and social form than occurs in the introduced range (*5*). Although the *b* allele is found only in the polygyne form in both ranges, some polygyne nests in Argentina contain egg-laying queens scored by SGPE as *BB* homozygotes, whereas only *Bb* queens occur in polygyne nests in the United States. This difference has been hypothesized to result from the presence in native polygyne ants of a "cryptic," functionally *b*-like allele that encodes a protein bearing the net charge of, and thus electrophoretically indistinguishable from, a *B* allele product (*22*). We tested this hypothesis by sequencing *Gp-9* in two monogyne queens scored by SGPE as *BB* homozygotes, two polygyne queens scored by SGPE as *Bb* heterozygotes, and two polygyne queens scored by SGPE as *BB* homozygotes, all from Argentina (*19*). The sequence

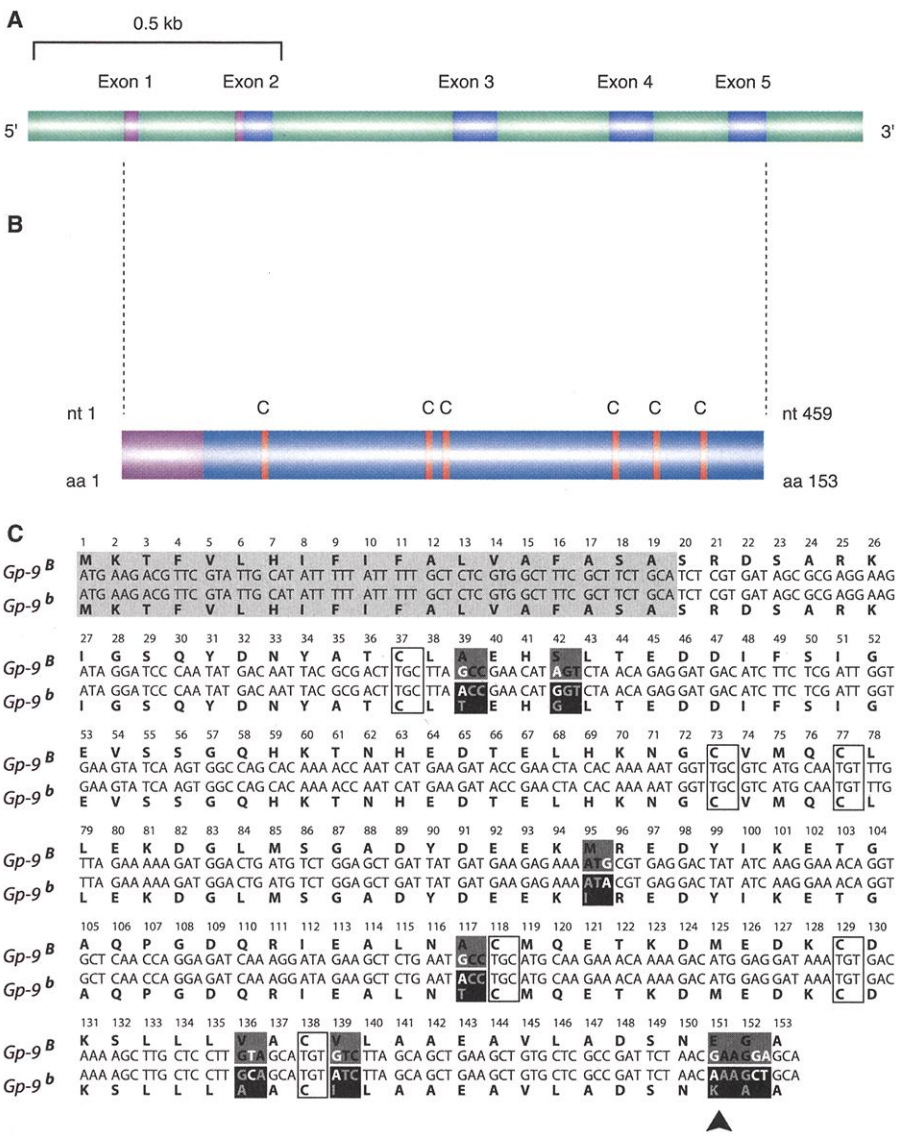

Fig. 1. Structure and sequences of *Gp-9* in fire ants. (**A**) Intron/exon structure of *Gp-9*. The signal sequence is shaded purple, and the remaining coding regions are shaded blue. (**B**) Structure of GP-9 precursor protein. The signal peptide is shaded purple, and the six cysteine residues characteristic of pheromone-binding proteins (PBPs) are shown in red. nt, nucleotide; aa, amino acid. (**C**) Coding region nucleotide and amino acid sequences (*38*) for the *B* and *b* alleles from *S. invicta* in the introduced range. The signal peptide is shaded light gray, and the six cysteine residues characterizing PBPs are enclosed in rectangles. Nucleotides that differ between the alleles are shown in white. The charge-changing amino acid substitution responsible for the different electrophoretic mobilities of the allelic proteins is indicated by an arrowhead.

data confirmed that the monogyne queens had only allele *B* and that each polygyne *Bb* queen had both the *B* and *b* alleles (*23*). The polygyne queens scored electrophoretically as *BB* homozygotes in fact carried two alleles—one a *B* allele (*Sinv.**B1***) and the other a unique allele, designated *Sinv.**b***′, that is more similar to *b* alleles than *B* alleles over its coding sequence (98.0% and 96.1% amino acid identity, respectively) yet bears the same charge-conferring amino acid as the *B* alleles at position 151 (Fig. 2) (*24*). These findings were verified by analyzing six additional queens of each of the three types with a polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) assay (*25*) in concert with SGPE. Confirmation of the existence of the cryptic *b*-like allele *Sinv.**b***′ in native polygyne queens constitutes powerful additional evidence that *Gp-9* is involved in the regulation of social organization in *S. invicta*.

We next attempted to sequence *Gp-9* in nine other native *Solenopsis* species, as well as in a species of the related myrmicine genus *Monomorium*, to establish the taxonomic range over which a homolog occurs (*26*). We were able to amplify and sequence the entire 2200-bp region in each of the *Solenopsis* fire ant species examined, but in the *Solenopsis* thief ant species chosen, we were able to sequence the gene only by using a reverse primer that anneals immediately downstream of the stop codon (*27*). Every predicted amino acid sequence from these other species featured a glutamic acid at position 151 (e.g., Fig. 3), consistent with the identical electrophoretic mobilities of these proteins and the product of the *B* alleles of *S. invicta* and suggesting a single recent origin of the charge-changing substitution in the *b* alleles of *S. invicta*. We were unable to amplify *Gp-9* in the *Monomorium* specimen, suggesting that a homolog in this ant, if it exists, has undergone extensive sequence divergence at potential primer binding sites.

We used the sequence data from the different *Solenopsis* species to reconstruct the evolutionary relationships of the *Gp-9* variants (Fig. 4). The fire ant sequences form a monophyletic group highly divergent from the thief ant sequence, the native North American fire ant sequences form a sister clade to the South American sequences, and the *S. interrupta* and *S. saevissima* sequences are basal within the South American fire ant clade, findings consistent with the classification of these ants (*28*) and with results from mitochondrial DNA sequence analyses (*29*). Most *Gp-9* sequences from the South American fire ants known to display polymorphism in social organization form sister clades, one containing the close relatives of the polygyny-inducing *b* alleles of *S. invicta* and the other containing the close relatives of the *B*

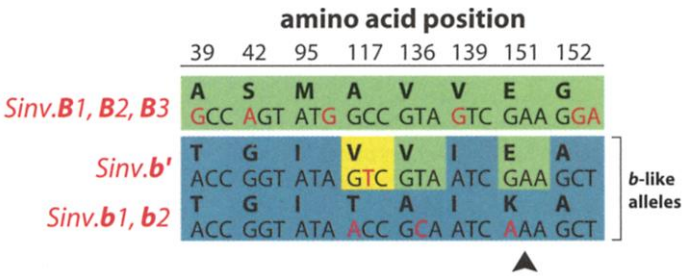

Fig. 2. Coding region nucleotide and amino acid variation for the alleles of *Gp-9* from *S. invicta* in the native and introduced ranges. The *b*-like alleles invariably are associated with polygyny. Three different *B* alleles and two *b* alleles were detected on the basis of their noncoding sequences (*21*, *23*). The charge-changing amino acid substitution in the *b* alleles is indicated by an arrowhead.

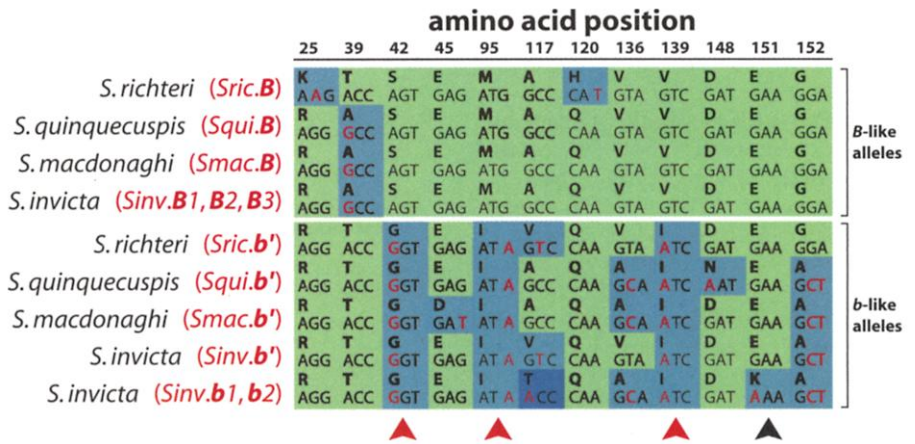

Fig. 3. Coding region nucleotide and amino acid variation for the alleles of *Gp-9* in the socially polymorphic fire ant species. Alleles are indicated by the name of the species in which they are found (black lettering), followed by the formal allele designations (red lettering). The charge-changing amino acid substitution in the *b* alleles of *S. invicta* is indicated by a black arrowhead. The amino acids distinguishing all *b*-like from all *B*-like allele products are indicated by red arrowheads.

alleles of *S. invicta*. As expected if alleles in the *b*-like clade induce polygyny, confirmed polygyne nests of *S. richteri*, *S. quinquecuspis*, and *S. macdonaghi* invariably contained individuals with these alleles (*26*). The *B*-like allele of *S. richteri* is the sister sequence to all other *B*-like and *b*-like alleles, suggesting that the ancestral *Gp-9* allele for the socially polymorphic clade was of the *B* type and, hence, that monogyne social organization preceded polygyny in the evolutionary history of South American fire ants (*S. interrupta* and *S. saevissima* are not known to exhibit polygyny, consistent with their possession of ancestral *B*-like alleles). The implied single origin of *b*-like alleles in these ants apparently predated the origins of most of the species, suggesting that the expression of polygyny in each was made possible by survival of the descendants of an ancestral *b*-like allele through sequential speciation events.

The availability of a gene phylogeny for *Gp-9* makes possible formal tests for the presence of selection during the evolutionary history of this gene. In particular, we wished to test the hypothesis that the *b*-like alleles, presumably integral to the polygyne social system of each species, are under different selective regimes than the *B*-like alleles, which must function in both social systems, with positive selection on *b*-like alleles in the polygyne environment having been instrumental in the divergence of the two allele types [e.g., (*5*, *7*, *16*)]. We inferred the ancestral nucleotide sequences for relevant interior nodes of the phylogeny (*30*) and examined all branches for evidence of selection by testing for differences in substitution rates between nonsynonymous and synonymous sites (*31*–*33*). As hypothesized, positive selection, signified by excess nonsynonymous substitutions, is statistically detectable only on branches within the *b*-like clade (Fisher's exact test, all $P < 0.05$), including the stem lineage of the clade and the *S. invicta* lineage that acquired the charge-changing lysine at position 151 (Fig. 4). Despite positive selection having acted periodically on various *b*-like alleles to drive their divergence from their *B*-like counterparts, all *b*-like alleles uniquely share the three amino acids G^{42}, I^{95}, and I^{139} (Fig. 3), suggesting that one or more of these is essential to the functional role of the gene product in inducing polygyny.

This study identifies a single gene with major effects on the regulation of complex social behavior. Analyses of *Gp-9* sequences from multiple *Solenopsis* species suggest that this genetic basis to social polymorphism is conserved in South American fire ants and

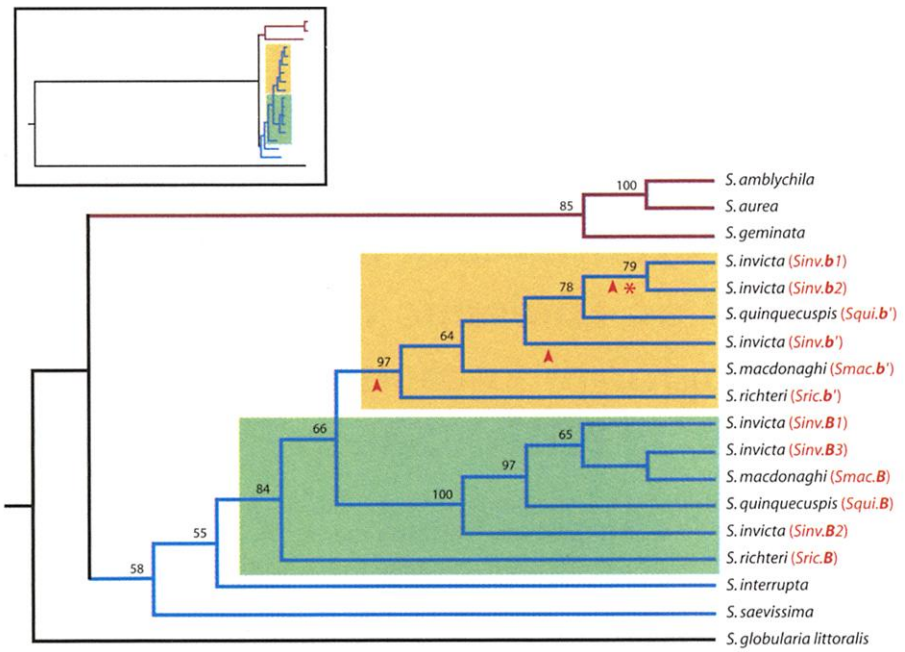

Fig. 4. Cladogram depicting the phylogenetic relationships of 18 *Gp-9* alleles found in 10 *Solenopsis* species (branch lengths reflecting uncorrected sequence divergence are shown in inset) (*39*). Each allele is indicated by the name of the species in which it is found (black lettering), followed by the allele designation (red lettering) in the case of the socially polymorphic South American fire ant species with multiple alleles. Alleles from native North American fire ant species are indicated by maroon branches, and those from South American species are indicated by blue branches. Alleles invariably associated with polygyny (*b*-like alleles) have branches highlighted with yellow background, and those known or assumed to be fixed in the monogyne forms (*B*-like alleles) have branches highlighted with green background. Branches for which episodes of positive selection were detected are indicated by red arrowheads; the branch on which the charge-changing amino acid was acquired is shown by an asterisk. Support for nodes obtained from 1000 bootstrap character resamplings is indicated for groups that appeared in ≥50% of the bootstrap trees (expressed as percentages). The consistency index for the tree is 0.922, the retention index is 0.821, and the homoplasy index is 0.078.

implicate positive selection as driving the divergence between the *b*-like alleles associated with polygyny and the alternate, *B*-like alleles common to both social forms. The product of *Gp-9* is a protein predicted to be a key molecular component in chemical recognition of conspecifics, making it likely that variation in *Gp-9* genotypes between the social forms leads to different abilities of workers to recognize and discriminate among potential egg-laying queens in a colony, the proximate behavioral mechanism by which queen number and social organization are regulated in fire ants. Nonetheless, given the diversity of phenotypic effects associated with *Gp-9* genotype and the likelihood that *Gp-9* occurs in a genomic region with reduced recombination (*5*, *22*), other genes of large effect in gametic disequilibrium with *Gp-9* may also be involved in regulating social organization. Future studies of the gene content of this region, combined with biochemical analyses of the gene products and phylogenetic analyses of the gene sequences, promise to yield insights into the genetic basis of social evolution while providing an integrative paradigm for studies of complex sociality.

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8. NH_2-terminal amino acid sequence of GP-9: The thorax of a monogyne queen from northern Georgia, United States, was homogenized in buffer, and the protein extract was separated electrophoretically in a starch gel (*27*). Proteins were blotted onto a polyvinylidene difluoride (PVDF) membrane and then stained with amido black; the band containing GP-9 was excised, and the protein was subjected to NH_2-terminal sequencing. Internal amino acid sequences of GP-9: Protein extracts of 10 monogyne queens were pooled and separated in a starch gel, and the gel fragment containing the GP-9 band was excised and macerated in buffer (*27*). The supernatant was concentrated, separated in an SDS–polyacrylamide gel electrophoresis gel, and blotted onto a PVDF membrane. After staining, the membrane area containing GP-9 was excised, and the protein was digested with the endoproteinase Lys-C. The resulting fragments were separated by high-performance liquid chromatography (HPLC), and the peptides represented by major recorded peaks were collected and sequenced.
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10. Partial nucleotide sequence: Total RNA isolated from several monogyne queens was reverse transcribed with AMV reverse transcriptase and oligo-$dT_{(18)}$ primer, and the cDNA transcript was PCR-amplified (*27*). The PCR product was gel purified and cloned, and then both strands were sequenced.
11. 3′ sequence: Total RNA was reversed transcribed with oligo-$dT_{(12\text{-}18)}$ primer, and the resulting cDNA was PCR amplified with this same primer and a gene-specific primer designed from sequences acquired with the degenerate primers (*27*). The reaction products were purified and subjected to a second round of amplification with an oligo-$dT_{(23)}$V reverse primer and a second, gene-specific forward primer. This product was gel purified, cloned, and sequenced. 5′ sequence: cDNA was synthesized from total RNA with a gene-specific primer (*27*). After purification, the 3′ ends were tailed with deoxyadenosine triphosphates with calf thymus terminal transferase. The tailed cDNA was PCR-amplified with the forward primer 5′ $CCATGGT_{(18)}$V 3′ and a gene-specific reverse primer. The reaction products were purified and subjected to a second round of PCR with the same forward primer, a different gene-specific reverse primer, and an increased annealing temperature. This product was gel purified, cloned, and sequenced.
12. The signal peptide sequence was determined by comparing the amino acid sequence of the NH_2-terminus of the mature protein with the amino acid sequence inferred from mRNA transcripts.
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14. The spacing of the cysteine residues in GP-9 is X_{36}–**C**–X_{30}–**C**–X_3–**C**–X_{41}–**C**–X_{10}–**C**–X_8–**C**–X_{15} (where X represents any amino acid), and in moth PBPs it is $X_{36\text{-}42}$–**C**–X_{30}–**C**–X_3–**C**–X_{42}–**C**–X_{10}–**C**–X_8–**C**–$X_{24\text{-}27}$.
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17. Total RNA was reversed transcribed from five egg-laying polygyne queens and five virgin monogyne queens (*19*) with an oligo-$dT_{(12\text{-}18)}$ primer (*27*). The resulting cDNA was PCR-amplified with gene-specific primers located in the 5′ and 3′ flanking sequences of *Gp-9*, and the amplification products were sequenced.
18. Virtually all egg-laying polygyne *S. invicta* queens from the United States genotyped by SGPE have been found to be $Gp\text{-}9^{Bb}$ heterozygotes (*5*). Homozygous *BB* queens of this form are destroyed by workers as they mature (*16*), whereas homozygous *bb* queens die of presumed intrinsic defects early in adult life (*5*, *34*).
19. The social form of colonies, determined initially in the field with well-established diagnostic criteria, was confirmed subsequently by examining worker genotype distributions at several polymorphic loci (*5*, *7*, *27*, *35*). Because nestmate fire ants often are close relatives, only one individual per nest was used for all analyses.
20. Genomic DNA was extracted and the *Gp-9* gene region, including the 170-bp 3′ untranslated region (UTR) as well as an additional 324 bp from the 3′ flanking region, was PCR amplified with proofreading DNA polymerase and two gene-specific primers (*27*). The reaction products were cloned, and a minimum of two clones were sequenced for each allele.
21. All polygyne queens had the identical *b* allele (designated *Sinv.b1*), which differs from all *B* alleles at noncoding regions by six nucleotide substitutions in the first intron and 3′ flanking region. Three different *B* alleles were discovered. The most common (*Sinv.B1*) was found in queens of both forms. A second, found in monogyne queens only (*Sinv.B2*), differed from the *Sinv.B1* allele by three substitutions in the fourth intron and 3′ flanking region, as well as a 3-bp deletion in the flanking region. The third *B* allele, found in polygyne queens only (*Sinv.B3*), differed from the *Sinv.B1* allele by a single substitution in the first intron.
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23. The Argentine *B* allele from both forms is identical to the most common *B* allele in the United States (*Sinv.B1*), whereas the Argentine *b* allele found in the polygyne form (*Sinv.b2*) differs from the *Sinv.b1* allele in the United States by a single substitution in the first intron.

24. At the coding regions, *Sinv.b'* differs from the other *b*-like alleles of *S. invicta* at four nucleotide positions, resulting in three amino acid differences (Fig. 2). Three of these four divergent *Sinv.b'* nucleotides are identical to the corresponding nucleotides in all *B* alleles, with the result that only six amino acids differ between *Sinv.b'* and the *B* alleles. None of these six amino acid differences is expected to produce a net charge difference (*Sinv.b'* and the *B* alleles share a glutamic acid at position 151), explaining why the protein product of *Sinv.b'* is electrophoretically indistinguishable from that of the *B* alleles. All noncoding regions of *Sinv.b'* are identical to *Sinv.b1*.
25. Differences in nucleotide sequence between the *B* and *b*-like alleles of *Gp-9* were used to design a diagnostic PCR/RFLP assay (*27*). Genomic DNA was amplified with gene-specific primers, and the resulting 828-bp product was purified and digested with Bsa AI. The allele-specific fragments produced were separated in a 1% agarose gel containing ethidium bromide and then visualized under ultraviolet light.
26. Single specimens for sequencing were obtained for the fire ant species *Solenopsis amblychila*, *S. aurea*, and *S. geminata* from the United States, the fire ant species *S. interrupta*, *S. macdonaghi*, *S. quinquecuspis*, *S. richteri*, and *S. saevissima* from Argentina and Brazil, the thief ant species *S. globularia littoralis* from the United States, and an unidentified species of the solenopsidine genus *Monomorium* from the United States. The specimens of *S. macdonaghi*, *S. quinquecuspis*, and *S. richteri* were obtained from known polygyne nests (*19*); all other fire ant specimens were from known or presumed monogyne nests. *Gp-9* sequences are deposited in GenBank under the accession numbers AF427889 through AF427906 and AF459414; the aligned sequences are presented in Web fig. 1 (*27*).
27. Supplementary material, including details of methods and complete aligned sequences, is available on *Science* Online at www.sciencemag.org/cgi/content/full/1065247/.
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31. Selection is implicated when the rate of nonsynonymous substitutions, which lead to amino acid changes, significantly exceeds (positive selection) or falls short of (negative selection) the rate of synonymous substitutions, which do not lead to such changes (*32*, *33*). Fisher's exact test of homogeneity was used to test $n/N = s/S$ along each branch under the null hypothesis of neutral evolution, where n and s are the numbers of nonsynonymous and synonymous substitutions per *Gp-9* sequence, respectively, and N and S are the numbers of potential nonsynonymous and synonymous sites (*32*). We pooled coding region synonymous sites with intron sites for these tests (*33*) after determining that there are no differences in substitution rates between them (Fisher's exact test, all $P > 0.09$). No episodes of negative selection were detected in this study.
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38. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
39. The tree was generated with the neighbor-joining algorithm (*36*) on the uncorrected pairwise sequence divergences over 2200 bp that include the *Gp-9* exons and introns, the 3′ untranslated region (UTR), and the 3′ flanking region (*27*, *37*). The strict consensus of five maximum parsimony trees obtained with the branch-and-bound method has a topology identical in its essential features to the neighbor-joining tree (*27*).

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The Potential Size and Duration of an Epidemic of Bovine Spongiform Encephalopathy in British Sheep

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Because there is a theoretical possibility that the British national sheep flock is infected with bovine spongiform encephalopathy (BSE), we examined the extent of a putative epidemic. An age cohort analysis based on numbers of infected cattle, dose responses of cattle and sheep to BSE, levels of exposure to infected feed, and number of BSE-susceptible sheep in the United Kingdom showed that at the putative epidemic peak in 1990, the number of cases of BSE-infected sheep would have ranged from fewer than 10 to about 1500. The model predicts that fewer than 20 clinical cases of BSE in sheep would be expected in 2001 if maternal transmission occurred at a rate of 10%. Although there are large uncertainties in the parameter estimates, all indications are that current prevalence is low; however, a simple model of flock-to-flock BSE transmission shows that horizontal transmission, if it has occurred, could eventually cause a large epidemic.

BSE in the United Kingdom was spread through feed containing meat and bone meal (mbm) contaminated with BSE-infected animal material (*1*). Sheep in the United Kingdom were also fed mbm, and it is known that sheep can be infected with BSE by the oral route (*2*). No field cases of sheep BSE have been observed, but it has been a concern for a number of years (*3*), and the Food Standards Agency of the UK government has recently demanded a comprehensive search for it (*4*), in part because sheep are the natural hosts of scrapie, a transmissible spongiform encephalopathy (TSE) that has clinical signs indistinguishable from BSE. If BSE is masquerading as scrapie in the national flock, two independent estimates show that the current number of BSE cases (i.e., sheep that live long enough to show signs of infection) in sheep is likely to be few. First, an extensive survey of UK flocks found no indication of an increase in scrapie incidence during the height of the cattle BSE epidemic (*5*, *6*). The sensitivity of this study implies that fewer than 200 flocks could have been acquiring a case of BSE per year at the peak. Second, in the late 1990s, of 156 brains taken from sheep purported to be infected with scrapie, none contained BSE, indicating that between 0 and 100 or at most 2% of yearly scrapie cases were actually BSE at that time (*4*).

Using a simple age cohort analysis, we examined the extent of a putative epidemic of BSE in British sheep and compared our results with these estimates. Our calculations are based on the cattle infection rate, the dose responses of cattle and sheep, their relative yearly consumption of mbm, and estimates of the number of BSE-susceptible sheep obtained from a survey of sheep PrP genotypes (*7*, *8*). In the year of the epidemic peak, the number of cases of BSE-infected sheep calculated is consistent with both of the existing estimates. Should there be horizontal transmission of BSE from sheep to sheep, a model of flock-to-flock BSE transmission shows that even if current cases are few, such an epidemic could be in its very early stages and a substantial epidemic in the future cannot be ruled out.

The susceptibility of sheep to BSE is strongly associated with the encoding of glutamine

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