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16. Tissues were dissected out and fixed for 2 hours on ice in phosphate-buffered saline (PBS), 1% formaldehyde, 0.2% glutaraldehyde, 0.02% NP-40, and 1 mM $MgCl_2$, washed twice in PBS and 0.05% bovine serum albumin, and then stained overnight at 37°C in PBS and 1 mM $MgCl_2$, 0.04% X-Gal, 5 mM potassium ferrocyanide, and 5 mM ferricyanide. Tissues were then washed, post-fixed in PBS and 4% paraformaldehyde, and processed for histological analysis. Sections were counterstained with eosin and hematoxylin.
17. The complete genomic structure of the mouse *whn* locus will be described (M. Schorpp *et al.*, in preparation).
18. The targeting construct was derived as follows. P1 recombinants spanning the *whn* locus [M. Nehls *et al.*, *Mamm. Genome* **6**, 321 (1995)] were partially digested with Sau 3AI, selected by size, and cloned into λKO [M. Nehls, M. Messerle, A. Sirulnik, A. J. H. Smith, T. Boehm, *Biotechniques* **17**, 770 (1994)], which supplies thymidine kinase genes at either end of the insert. Recombinants containing exon 3 were analyzed for inserts in which the Sfi I site was at a reasonable distance from one end of the insert, to facilitate subsequent polymerase chain reaction (PCR) screening. In the clone used here (32), the Sfi I site was located about 1.8 kb downstream of the 5′ end of the homologous sequences and about 4.2 kb upstream of the 3′ end of the homologous sequences. Phage DNA was concatamerized by ligation at its cos ends and then cut with Sfi I. The Sfi I ends were modified by addition of Bam HI adapter oligonucleotides and this DNA subsequently ligated to a Bam HI cassette containing a promoterless β-galactosidase gene preceded by an internal ribosomal entry sequence followed by a neomycin resistance gene (7). The ligation was packaged in vitro, and desired recombinants were selected by plaque hybridization, converted into plasmid form, and linearized with Not I. This linear molecule was then ligated to a self-complementary Not I–compatible, phosphorylated oligonucleotide (5′-GGCCTCCGGTACATGATCGAGGGGACTGACAAGACGGCCAGTCCTCGATCATGTACCGGA-3′) to seal the ends of the linear fragment. This treatment enhances the stability of transfected DNA, improves positive-negative selection, and increases the frequency of homologous recombination (M. Messerle and T. Boehm, in preparation). The targeting construct was transfected into CJ7 cells [P. J. Swiatek and T. Gridley, *Genes Dev.* **7**, 2071 (1993)], and clones resistant to G418 and gancyclovir were analyzed by PCR and Southern (DNA) blotting for homologous recombination. Of 25 colonies tested, 23 were homologous integrants. Mice heterozygous for the insertional mutation were derived as described [A. Warren *et al.*, *Cell* **15**, 45 (1994)].
19. For flow cytometric analysis, cell suspensions of mesenteric lymph nodes and spleen were depleted of erythrocytes by brief NH_4Cl treatment and then triple stained with the following antibody combinations: antibodies to α/β TCR–fluorescein isothiocyanate (FITC) (*15*), CD4-phycoerythrin (PE), CD8–Red 613 (both from Gibco-BRL), or α/β TCR-FITC, B220-PE (Pharmingen, San Diego), and CD2-biotin (*16*)–streptavidin–Red 670 (Gibco-BRL). Cells were analyzed with a FACScan cytometer (Becton and Dickinson, Heidelberg, FRG). Lymphoid cells were gated by appropriate forward and side scatter, and 10,000 events were analyzed with Lysis-II software. The cell yield from $whn^{+/+}$, $whn^{+/-}$, and $whn^{-/-}$ mice was 1.2×10^7, 0.7×10^7, and 0.4×10^7 for mesenteric lymph nodes, and 4.6×10^7, 4.0×10^7, and 6.0×10^7 for splenocytes, respectively. The frequency of CD2-positive cells ranged between 93 to 99% but was reduced to 65% in spleen cells of $whn^{-/-}$ mice, indicating an increase of nonlymphoid cells.
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21. Frozen sections (3 μm) were acetone-fixed, pretreated with human γ globulin (Venimmun, Behringwerke AG, Marburg, FRG), and stained with the following antibody combinations: (i) rabbit immunoglobulin G (IgG) anti–β-galactosidase (Organon Teknika, West Chester, PA), goat antibody to rabbit IgG-Cy3 (Jackson, Avondale, PA) supplemented with 5% normal goat serum, mouse antibody to pan-cytokeratin (clone Lu-5, Dianova GmbH, Hamburg, FRG), and goat antibody to mouse IgG1-FITC (Southern Biotechnology Association, Birmingham, AL); (ii) rabbit-IgG anti–β-galactosidase, goat antibody to rabbit IgG-Cy3 supplemented with 5% normal goat serum, ER-TR4 (*13*) or ERTR5 (*13*) culture supernatant, $F(ab')_2$ goat antibody to rat IgG-biotin (Jackson) supplemented with normal goat and rabbit sera at 5% each, and streptavidin-FITC (Boehringer Mannheim, FRG). Stained sections were photographed with an Axiophot microscope (Zeiss) with Fujichrome Provia 400 film.
22. We thank T. H. Rabbitts for advice, I. Schuster, S. Höflinger, G. King, and T. Langford for expert technical help, H. Schrewe for CJ7 cells, and W. van Ewijk for antibodies. These studies were supported by a grant from the Deutsche Forschungsgemeinschaft (Bo1128/2-2). A.J.H.S. is supported by the Association of International Cancer Research.

31 January 1996; accepted 25 March 1996

Chemical Usurpation of a Nest by Paper Wasp Parasites

Anne-Geneviève Bagnères,* Maria Cristina Lorenzi, Georges Dusticier, Stefano Turillazzi, Jean-Luc Clément

The paper wasp *Polistes atrimandibularis* is an obligatory social parasite of another *Polistes* species, *P. biglumis bimaculatus*. To control the host nest, the parasite sequentially changes the composition of its chemical signature, the cuticular hydrocarbons, during the colonial cycle. Gas chromatography–mass spectrometry of the cuticular hydrocarbons at every stage of the cycle showed that the parasite can switch on and off an entire chemical family, namely, the unsaturated hydrocarbons. In this way the parasite can match the host signature at a critical moment of the colonial cycle.

Having no worker caste and being incapable of nest building, *P. atrimandibularis* is an obligatory social parasite. During the short 4-month summer colonial cycle of the host, a *P. atrimandibularis* queen usurps a nest of a *P. biglumis bimaculatus* foundress (*1*, *2*). Nest invasion occurs within the span of a few hours, during which the initially unaggressive parasite becomes increasingly dominant and begins egg laying. In contrast the host queen, at first very aggressive, becomes subdued (*3*). To date there has been no satisfactory explanation for the ease with which *P. atrimandibularis* controls the host colony.

In insects, environmental perception often relies heavily on olfaction, gustation, or both. In social wasps, nestmate–non-nestmate discrimination depends on odors (*4*). These findings led us to suspect that chemical mimicry could be involved in nest usurpation by *P. atrimandibularis*. Like other social insects, the host *P. biglumis bimaculatus* has a chemical signature that facilitates recognition between colony members (*5*). This species- and colony-specific signature depends mainly on cuticular hydrocarbon components (*6*), which are dependent on environmental and physiological factors (*7*). Plasticity or camouflage of cuticular hydrocarbon patterns has already been reported in insects living in natural and artificial heterospecific colonies (*8*).

The purpose of this study was to better understand the integration mechanism by comparing the chemical signature of the parasite, the host, and their descendants collected in the field at different times of the colonial cycle. We extracted almost 80 different cuticular products from the two species, all of which were hydrocarbons ranging in chain length from C_{23} to C_{37}. Data for mathematical and statistical analysis were obtained by gas chromatography (GC) on individual extracts, and the identification of hydrocarbons was achieved by GC–mass spectrometry (GC-MS) on pooled extracts (*9*). Considerable variations in cuticular hydrocarbons of individuals were noted during the short colonial cycle (*10*).

Polistes atrimandibularis queens, fertilized in the previous summer, begin searching for a host comb about 1 month after solitary nest founding in early June by the host queen. At this time, just before invasion (late June), the cuticular signatures of the two species are distinct (Fig. 1A). The signature of *P. atrimandibularis* females is extremely rich in unsaturated hydrocarbons,

A.-G. Bagnères, G. Dusticier, J.-L. Clément, CNRS UPR 9024, Laboratoire de Neurobiologie-Communication Chimique, 31, Chemin Joseph Aiguier, 13402 Marseille Cedex 09, France.
M.-C. Lorenzi, Dipartmento di Morfofisiol Veterinaria, Università di Torino, Viale Mattioli 25, 10125 Turin, Italy.
S. Turillazzi, Dipartmento di Biologia Animale é Genetica, Università di Firenze, Via Romana 17, 50125 Florence, Italy.
*To whom correspondence should be adressed.

that is, alkenes (Fig. 1C), whereas the signature of *P. biglumis bimaculatus* foundresses is composed only of saturated compounds (*11*). Soon after nest invasion in late June, all the specific unsaturated hydrocarbons disappear from the cuticle of the parasite, and some of the saturated products characteristic of the host *P. biglumis bimaculatus* appear (*12*). At this time the two signatures do not match.

By the end of July, at the time of the emergence of the descendants of *P. biglumis bimaculatus* (workers and reproductives), the signatures of the two queens are indistinguishable (Fig. 1B) (*13*). The results of multivariate principal component analysis (PCA) (*14*) show several *P. atrimandibularis* queens from different nests blending in with *P. biglumis bimaculatus* females (Fig. 2). This qualitative and quantitative modification takes place despite the natural drift (*15*) that occurs in the signature of *P. biglumis bimaculatus* regardless of whether or not the nest is invaded. Selected ion research by GC-MS reveals that the *P. atrimandibularis* queen no longer produces unsaturated cuticular compounds but rather picks up or produces the same saturated products as the host. Even the quantitative profile of the diagnostic ions of long-chain methylalkanes is similar in the two species (Fig. 1D) (*16*).

In late August and September, 2 to 3 months after invasion, the parasitized colony has a complex structure. *Polistes atrimandibularis* reproductive males and females have begun to emerge and outnumber the descendants of the host queen. Although the chemical signature of *P. atrimandibularis* female offspring is quantitatively similar to the saturated signature of the queen in late June (Fig. 2), GC-MS analysis showed that qualitatively it is intermediate between the signature observed before nest invasion and that observed in July, with a number of peaks containing a mixture of saturated and unsaturated products (Fig. 1E). The *P. biglumis bimaculatus* signature continues to display the characteristic saturated specific profile and never acquires unsaturated products from the parasite nestmates (Fig. 1E) (*17*). Host workers take care of emerging *P. atrimandibularis* despite the fact that they have a different specific signature (*18*). The *P. biglumis bimaculatus* foundress usually leaves the nest during August, but the *P. atrimandibularis* queen generally stays. At the end of the colonial cycle, the parasite queen can be chemically distinguished from her descendants: She presents the preinvasion signature whereas her offspring present an intermediate alkene-rich signature (*19*). Extracts from the walls of nests collected at this time differ, depending on whether the comb was parasitized; thus, *P. atrimandibularis* descendants emerge in a nest that already has a part of their unsaturated odor (*20*). At the end of the summer, descendants of both species leave the nests to mate. The following summer, after hibernation, females begin a new colonial cycle.

The variations in peak 11 illustrate the biochemical phenomena that take place in

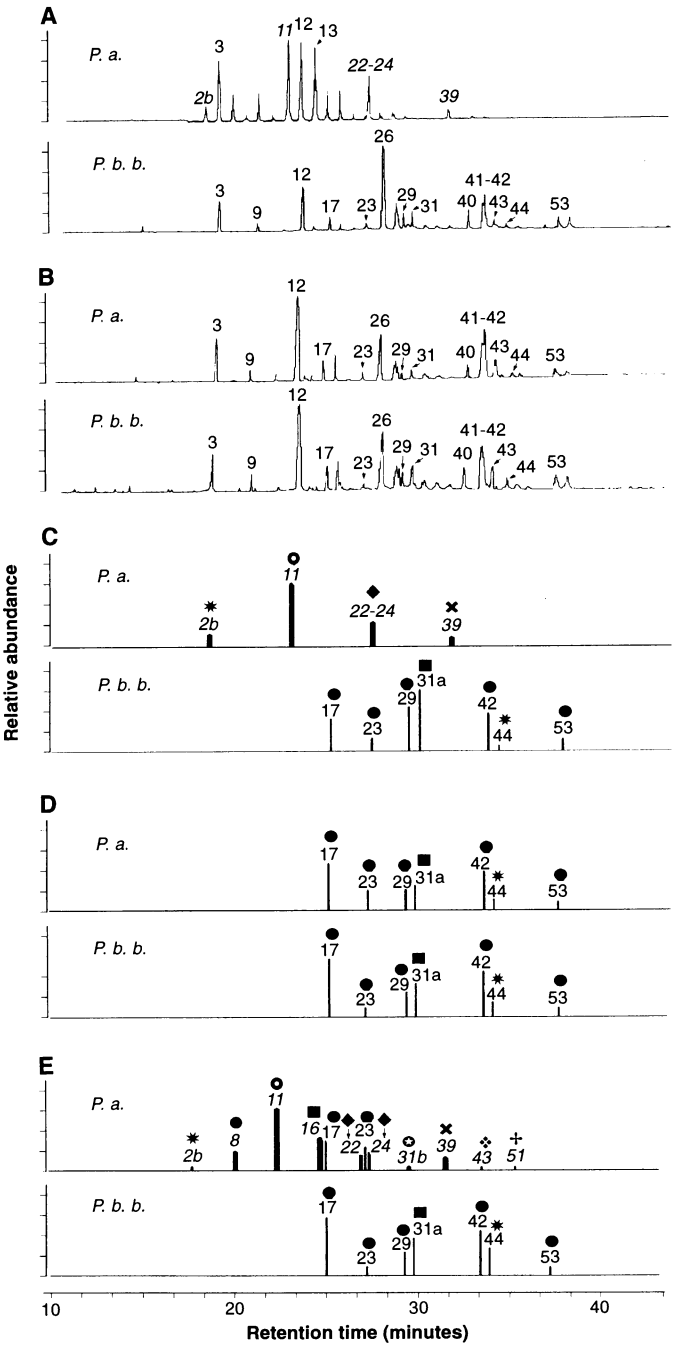

Fig. 1. Changes in the chemical signature of the two species during the colonial cycle. Peaks on all panels are indicated in relative abundance (*y* axis) and have been aligned by retention time (*x* axis). Chemical changes can be seen in the total ion chromatograms (TIC) of the two species (*P. atrimandibularis*, above, and *P. biglumis bimaculatus*, below), before (**A**) and during (**B**) cohabitation. Changes were also detected by GC-MS with selected ion chromatograms (SIC) of the two species: (**C**) SIC before usurpation, (**D**) SIC during chemical usurpation, and (**E**) SIC of parasite and host offspring at the end of the cycle. In *P. atrimandibularis* wasps with unsaturated products [(C) and (E) (even trace amounts)], the following ions respond positively (peak numbers and corresponding identification in parentheses): *m/z* of 350, ✱ (*2b* = 9-C25:1, a 25–carbon atom alkene with the double bond at position 9); *m/z* 378, **O** (*11* = 10+9-C27:1, a mixture of alkenes with the double bond at either position 10 or 9); *m/z* 406, ◆ (*22,24,25* = 12+10+9-C29:1); and *m/z* 434, ✖ (*39* = 14+9-C31:1) in all cases, and in addition *m/z* 364, ● (*8* = 10-C26:1); *m/z* 392, ■ (*16* = 10-C28:1); *m/z* 420, ✪ (*31b* = 14-C30:1); *m/z* 448, ❖ (*43* = C32:1); and *m/z* 462, ✣ (*51* = C33:1) at the end of the cycle [see *P.a.* of (E)]. In wasps with no unsaturated products (or a mixture) [see P.b.b. panels of (C) through (E)], some of the aforesaid ions responding positively were diagnostic ions for different methylalkanes including *m/z* 350, 364, and 392. Ion *m/z* 350 (✱) responds positively because it is one of the diagnostic ions for 9(11 or 13 or 17)-dimethylC31 (peak 44), the corresponding ion being *m/z* 140/1. Ion *m/z* 364 (●) corresponds to the following five hydrocarbons: 3-methylC27 (peak 17), which has the additional characteristic ions *m/z* 56/7, 365, 379(M-15); 2-methylC28 (peak 23), which has the additional characteristic ions *m/z* 365, 393(M-15) and the same retention time as nonacosenes (*22–24*); 5-methylC25 (peak 29), which has the additional characteristic ion fragments *m/z* 84/5, 365, 403(M-15); 7-methylC31 (peak 42), which has the additional characteristics ions *m/z* 112, 365, 435(M-15); and 9-methylC33 (peak 53), which also has the additional diagnostic ions *m/z* 140/1, 365, 463(M-15). Ion *m/z* 392 (■) corresponds to one of the characteristic ions of 3-methylC29 (peak 31a) with *m/z* 56/7, 393, and 407(M-15), this peak having the same retention time as 14-C30:1(*31b*) in our chromatography column. Diagnostic ions of unsaturated products are shown by thick lines with italicized numbers indicating the peak. Diagnostic ions of saturated products are shown by thin lines, the number is not in italics.

the cuticle of the parasite. The proportion of methylalkanes increases during the first phase of the cycle then dwindles off but never disappears. Alkenes are more strictly regulated as evidenced by their complete disappearance from the cuticle of the parasite and then reappearance. At the end of the colonial cycle the offspring signature includes additional unsaturated products (Fig. 1E) as if there was a reinforcement of the *P. atrimandibularis* alkene signature.

The present findings cast the cuticle in a much larger role than as a simple tegumental barrier, or even as a surface allowing recognition, a concept that was revolutionary only 10 years ago (*21*). This study demonstrates that the cuticle and its associated tissues and cells can act as a finely controlled system capable of starting or stopping synthesis (or release), of internalization, and perhaps even of storage of hydrocarbons. This system appears to have a mechanism that allows rapid regulation of the supply of products. Because the enzymological kinship of this parasite does not appear to be greater than between any two randomly selected *Polistes* species (*22*), it can be speculated that the parasite has an inherent chemical flexibility.

One hypothesis to explain our findings is a hormonal process that involves selective inhibition or stimulation (or both) of the genetic control of certain enzymes in the hydrocarbon metabolic pathways (*23*). Alternatively, there may be cessation of endogenous hydrocarbon production (possibly accompanied by degradation or internalization of the chemical signature of the parasite), followed by adsorption of the hydrocarbons of the host. Either of these processes would allow the parasite queen to adapt her specific signature to that of the host. The system may be triggered, under hormonal or neurohormonal control, by olfactory-gustatory perception of host hydrocarbons, or by a genetically programmed response linked to the endocrine cycle.

This usurpation strategy encompasses the full range of integration processes and provides an unprecedented example of a pheromonal tactic in which chemical changes and behavior are closely linked in four practically monthly steps. But why is the chemical signature of *P. atrimandibularis* so different from that of the host? In most parasite species the energy-consuming process of complete chemical disruption has been eliminated by evolutionary modifications in the basic signature. A possible explanation might be that this difference of signature serves another purpose such as stimulation of secretion of hormones necessary for parasite reproduction or fast establishment of dominance. This very powerful adaptive process, which can be compared to a chemical mystification, is crucial to the survival and reproductive success of the parasite.

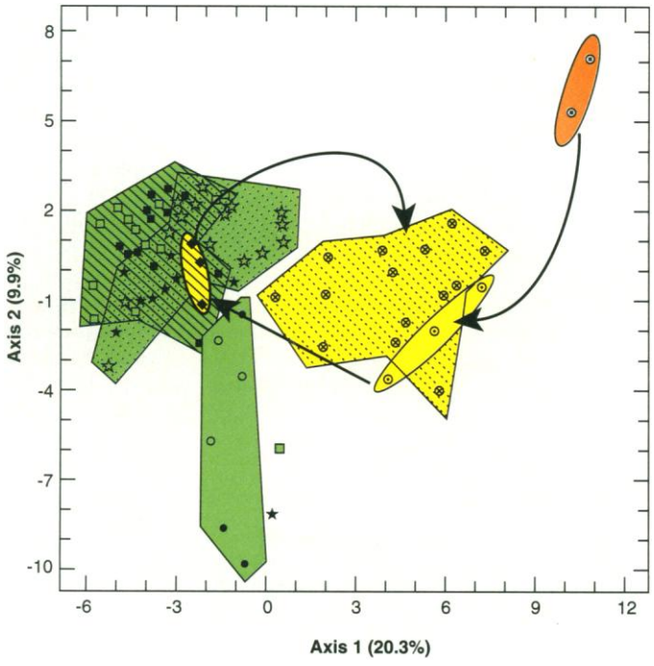

Fig. 2. PCA performed on individuals from *P. atrimandibularis* (yellow) and *P. biglumis bimaculatus* (green) from parasitized and nonparasitized colonies. Analysis was carried out on 78 quantitative variables (the relative proportions of cuticular hydrocarbons) automatically normalized, from 77 wasps (*9*). The first two axes account for 30.2% of the variation (20.3% for the first and 9.9% for the second). Axis 1 mainly separates individuals according to species, whereas axis 2 separates individuals according to the month of collection (June, plain areas; July, cross-hatched areas; and August, dotted areas). *Polistes atrimandibularis* from June (⊙) and August (⊗) are together on the positive side of axis 1, whereas *P. atrimandibularis* from July (◆) are on the negative side with *P. biglumis bimaculatus* from June (parasitized, ●; nonparasitized, green ○), July (parasitized, ■; nonparasitized, green □), and August (parasitized, ★; nonparasitized, green ☆). *Polistes atrimandibularis* females before invasion (⊜) (orange) are on the same side as those in June and August; however, because they are off center they are separated from the other two by axis 2. The black arrows indicate the cuticular cycle of *P. atrimandibularis*.

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5. *Polistes biglumis bimaculatus* has a specific and colonial chemical signature [M.-C. Lorenzi, A.-G. Bagnères, J.-L. Clément, S. Turillazzi, *Insectes Soc.*, in preparation] that, unlike that of *P. atrimandibularis*, does not depend on social environment, as can be seen in this work.
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9. GC was performed on individual pentane extracts (1 ml for 75 s) from 77 different insects. Twenty-four *P. biglumis bimaculatus* and 20 *P. atrimandibularis* were taken from 10 parasitized nests (three in June, three in July, and four in August). Thirty-one *P. biglumis bimaculatus* were taken from 12 unparasitized nests (three in June, three in July, and six in August). Two *P. atrimandibularis* females were taken before invasion. GC analysis was performed on a Delsi-Nermag 200 DN device, coupled to an Enica 31 integrator and equipped with a flame ionization detector (FID), a Chrompack capillary column (25 m, 0.25-mm internal diameter, CPSil5 WCOT 0.25-μm stationary phase), and a split-splitless injector (splitless mode 15 s), the carrier gas (helium) being at 1 bar. The temperature was programmed from 70° to 150°C at a rate of 30°C/min, then 150° to 320°C at 5°C/min (isothermal 5 min). Each integrated peak was entered into a Lotus spread sheet program and readjusted with the following coefficient: $K = 0.042 \times$ (number of carbons) + 0.11. The percentage and quantity per milligram of insect were calculated with n-C_{19} as the internal standard. Statgraphics software was used for PCA. For GC-MS, 139 individual samples (the 77 GC samples plus 62 from individuals of the same or other nests) were pooled in 30 GC-MS samples by species, sex, month, and nest. Analyses at the end of the cycle were performed with two pools: *P. atrimandibularis* queens and descendants. Hydrocarbons were identified with a Hewlett-Packard system with a GC 5890 II coupled to a 5989A MS

Engine mass detector controlled by a Chemstation HPUX. Analyses were performed after electron impact (EI-GC-MS, 2 bars He, 70 eV) with a program of scanned masses of mass-to-charge ratio (*m/z*) from 45 to 600 and after chemical ionization (CI-CH_4^+, 1.5 bar He, 150 eV), scanning from 100 to 600 *m/z*. The capillary column and temperature program were the same as those used for GC. Injection was performed in the splitless mode for 1 min. Analysis was done either by total ion chromatogram or selected ion chromatogram.

10. Peak 11, which often appears in Fig. 1, was chosen as a reference to illustrate step by step the complexity of the qualitative and quantitative changes that occur in the chemical signature of *P. atrimandibularis*.
11. In *P. biglumis bimaculatus*, peak 11 contains only 2-methylhexacosane and accounts for 0.76% of the signature mixture (relative proportion). Conversely, in *P. atrimandibularis* peak 11 is made up mainly of a combination of heptacosene isomers and the 2-methylhexacosane and accounts for 31% of the mixture. The proportion of the 2-methylhexacosane is low.
12. At this time peak 11 accounts for 1.1% of the cuticular mixture in *P. biglumis bimaculatus* foundresses and 6.46% in the *P. atrimandibularis* queen and is composed only of 2-methylhexacosane in both species.
13. The *P. biglumis bimaculatus* descendants (workers and reproductives) cannot distinguish between their mother and the heterospecific parasite. They work for the parasite and contribute to her reproductive success. In contrast, they recognize a conspecific parasite and refuse to cooperate with her.
14. PCA displays different normalized variables (vectors) on a single plane, accounting for maximum dispersion. This unbiased representation allows one to distinguish or associate individuals (wasps) according to their degree of similarity using the relative proportions of the different quantitative variables (cuticular hydrocarbons).
15. It is known that cuticular mixtures from a colony, or part of a colony, change continuously over time. These changes are controlled by a dynamic system of regulation that induces a chemical drift in the whole group of insects and probably contributes to establish the colonial odor of social insects [E. Provost, G. Rivière, M. Roux, E. D. Morgan, A.-G. Bagnères, *Insect Biochem. Molec. Biol.* **23**, 945 (1993)].
16. At the time of chemical usurpation, peak 11 is still composed only of 2-methylhexacosane and accounts for a similar relative proportion in the two species, that is, 0.58% in *P. biglumis bimaculatus* and 1.13% in *P. atrimandibularis*.
17. Peak 11 follows the same pattern: It returns to the same proportions as in June, that is, 1.06% for *P. biglumis bimaculatus* and 6.4% for *P. atrimandibularis* (including both the 2-methylhexacosane and the heptacosenes).
18. Comparable observations have been made in termites in which soldiers are fed by workers despite differences in caste signature [A.-G. Bagnères, thesis, Paris 6 University (1989)], and in slave-making ants in which the latter are fed by slaves taken at a larval stage, despite differences in specific signatures [C. Habersetzer and A. Bonavita-Cougourdan, *Physiol. Entomol.* **18**, 160 (1993)].
19. Peak 11 accounts for 1.02% of the 2-methylhexacosane in *P. biglumis bimaculatus* female descendants, 6.13% of a mixture of 2-methylC26 and heptacosenes in *P. atrimandibularis* female descendants, and 30.3% of the heptacosenes in the *P. atrimandibularis* queen.
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21. In the present discussion the term cuticle means integument that is the cuticle plus associated epidermal cells and tissue including lipophorin (transport proteins). Oenocytes that are usually associated with the epidermal layer are currently thought to be the most likely site of biosynthesis of cuticular hydrocarbons carried to the epicuticular layer through pore and wax canals [D. R. Nelson *et al.*, in *Insect Lipids: Chemistry, Biochemistry and Biology*, D. W. Stanley-Samuelson and D. R. Nelson, Eds. (Univ. Nebraska Press, Lincoln, NE, and London, 1993), pp. 271–315; G. Blomquist *et al.*, *ibid.*, pp. 317–351; X. Gu, D. Quilici, P. Juarez, G. J. Blomquist, C. Schal, *J. Insect Physiol.* **41**, 257 (1995)].
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24. We thank A. Bonavita-Cougourdan, R. Vander Meer, and K. Espelie for their useful comments on the initial draft, A. Boyer and A. Tirard for their help on the figures, and A. Corsini, M. Renucci, and C. Ronin for work on this manuscript.

10 November 1995; accepted 8 March 1996

An Enhanced Immune Response in Mice Lacking the Transcription Factor NFAT1

Steven Xanthoudakis, Joao P. B. Viola, Karen T. Y. Shaw, Chun Luo, James D. Wallace,* Patricia T. Bozza, Tom Curran,† Anjana Rao†

Transcription factors of the NFAT family are thought to play a major role in regulating the expression of cytokine genes and other inducible genes during the immune response. The role of NFAT1 was investigated by targeted disruption of the NFAT1 gene. Unexpectedly, cells from NFAT1$^{-/-}$ mice showed increased primary responses to *Leishmania major* and mounted increased secondary responses to ovalbumin in vitro. In an in vivo model of allergic inflammation, the accumulation of eosinophils and levels of serum immunoglobulin E were increased in NFAT1$^{-/-}$ mice. These results suggest that NFAT1 exerts a negative regulatory influence on the immune response.

The response of the immune system to antigen is coordinated by an interacting network of transcription factors that dictate expression of effector proteins such as cell surface receptors and cytokines (*1*, *2*). The recently identified NFAT family of transcription factors is thought to play a critical role in this process (*3*). NFAT DNA binding activity has been detected in nuclear extracts of antigen-stimulated T cells, B cells, mast cells, and natural killer (NK) cells (*4*, *5*), and NFAT binding sites have been identified in the promoter and enhancer regions of many genes encoding immunoregulatory proteins (*4*, *6*). The NFAT family comprises several structurally related proteins that are encoded by at least four distinct genes (*7*–*9*). The best characterized member of the NFAT family, NFAT1 (formerly NFATp) (*7*, *8*), is expressed constitutively as a cytoplasmic phosphoprotein in resting immune system cells (*10*, *11*); upon stimulation, it is rapidly dephosphorylated and translocated to the nucleus by way of a calcium-calcineurin–dependent pathway (*11*, *12*). Individual NFAT proteins may be able to substitute for one another in regulating expression of certain target genes, because each can bind cooperatively with Fos and Jun to DNA and activate transcription of the interleukin-2 (IL-2) promoter in transient transfection assays (*8*, *9*). However, the differential expression of NFAT proteins in tissues and the differences in their binding preferences for recognition sites in cytokine genes (*9*) suggest that each NFAT protein may control the expression of a distinct set of genes in vivo.

To study the unique functions of NFAT1 in vivo, we generated mutant mice carrying a disrupted NFAT1 allele (*13*). The targeting vector was designed to delete most of an exon encoding amino acids near the NH_2-terminus of the DNA binding domain (Fig. 1, A and B). A short segment of conserved amino acids encoded by this exon has been shown to be critical for DNA binding activity (*14*). Reverse transcriptase polymerase chain reaction (RT-PCR) anal-

S. Xanthoudakis and J. D. Wallace, Neurogenetics Program, Department of CNS Research, Hoffmann–La Roche, Nutley, NJ 07110, USA.
J. P. B. Viola, K. T. Y. Shaw, C. Luo, A. Rao, Division of Cellular and Molecular Biology, Dana-Farber Cancer Institute and Department of Pathology, Harvard Medical School, Boston, MA 02115, USA.
P. T. Bozza, Harvard Thorndike Laboratory and Charles A. Dana Research Institute, Department of Medicine, Beth Israel Hospital, Harvard Medical School, Boston, MA 02115, USA.
T. Curran, Department of Developmental Neurobiology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA.
*Present address: Lexicon Genetics, 4000 Research Forest Drive, The Woodlands, TX 77381, USA.
†To whom correspondence should be addressed.