Candidate odorant receptors from the malaria vector mosquito *Anopheles gambiae* and evidence of down-regulation in response to blood feeding

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Edited by John H. Law, University of Arizona, Tucson, AZ, and approved October 11, 2001 (received for review August 16, 2001)

Olfaction plays a major role in host preference and blood feeding, integral behaviors for disease transmission by the malaria vector mosquito *Anopheles gambiae* sensu stricto (henceforth *A. gambiae*). We have identified four genes encoding candidate odorant receptors from *A. gambiae* that are selectively expressed in olfactory organs, contain approximately seven transmembrane domains, and show significant similarity to several putative odorant receptors in *Drosophila melanogaster*. Furthermore, one of the putative *A. gambiae* odorant receptors exhibits female-specific antennal expression and is down-regulated 12 h after blood feeding, a period during which substantial reduction in olfactory responses to human odorants has been observed. Taken together, these data suggest these genes encode a family of odorant receptors in *A. gambiae*, whose further study may aid in the design of novel antimalarial programs.

Olfaction plays a critical role in many insect behaviors, including host preference selection, among agricultural pests and disease vectors (1, 2). These vectors include several species of mosquitoes that transmit numerous parasitic and viral diseases, including malaria, dengue, West Nile encephalitis, and yellow fever. Although all of these diseases pose significant threats to human health, malaria, which is transmitted by female adults of several species of anopheline mosquitoes, remains one of the leading causes of worldwide morbidity and mortality. The World Health Organization estimates that, in Africa alone, malaria is responsible for more than two million deaths per year, including one million children younger than 5 years of age (3). Because of the importance of host preference in establishing the overall vectorial capacity in this system, a molecular analysis of mosquito olfaction may provide opportunities for reducing the incidence of disease transmission. As a first step in this process, the cloning and characterization of components of the olfactory signal transduction cascade from *A. gambiae* will facilitate molecular and biochemical studies of this mosquito's olfactory processes. Ultimately, these efforts may lead to the design of novel methods of disrupting vector–host interactions and thereby reduce the vectorial capacity of these insects.

Olfactory signal transduction is widely conserved across a broad spectrum of organisms, including mammals, fish, crustaceans, nematodes, and insects (reviewed in ref. 1). The olfactory signaling cascade is initiated by seven-transmembrane-domain G-protein-coupled receptors (GPCRs) that either directly or indirectly bind odorants and, in turn, with heterotrimeric G proteins, activate downstream effector enzymes such as adenylate cyclase and phospholipase C. Second messengers are produced that modulate the opening of specifically gated channels and induce the depolarization or hyperpolarization of olfactory neurons (4, 5). The cloning of the GPCRs involved in this cascade, known as odorant receptors (ORs), has facilitated the molecular analysis of receptor–odorant interactions both in rats and in the nematode *Caenorhabditis elegans* (6, 7).

By using a variety of molecular and informatics-based approaches, numerous putative ORs have been identified from a variety of vertebrates (4). In addition, OR-encoding genes have been isolated from two invertebrates, *C. elegans* (8) and *D. melanogaster* (9–12). Because the fundamental molecular nature of ORs is conserved across these phyla, which include the dipteran *D. melanogaster*, we postulated that a similar family of seven-transmembrane-domain GPCRs would also mediate odorant signaling in *A. gambiae*.

Methods

Screening of *Anopheles gambiae* Genome Project. *A. gambiae* sensu stricto (G3 strain) bacterial artificial chromosome (BAC) sequences were transferred from Genoscope (http://www.genoscope.cns.fr/externe/English/Projets/Projet_AK/organisme_AK.html) to a local database. The local database was then analyzed for sequences similar to ORs in *Drosophila melanogaster* (DORs) by a modified tBLASTn algorithm (13).

Sequencing of *A. gambiae* BAC Clones. BAC clones displaying significant homology to DOR sequences were either directly sequenced or subcloned into pBluescript II KS(+) (Stratagene) and sequenced. Sequencing was performed with an ABI 377 automated sequencer using Big-Dye chemistry (PE Biosystems, Foster City, CA) with custom primers.

Phylogenetic Analysis. The four *A. gambiae* proteins were aligned with all 61 DORs, and 32 representative *D. melanogaster* gustatory receptors to serve as an outgroup, using CLUSTALX v1.8 (14) with minor manual adjustments. Phylogenetic analysis was performed with neighbor joining implemented by PAUP* v4.0b4 (15), with the distances corrected by using maximum-likelihood estimation and the BLOSUM62 matrix in TREE-PUZZLE v4.0.2 (16). Bootstrap analysis used 1,000 replications.

Reverse Transcription (RT)-PCR. Total RNA was reverse transcribed by using oligo(dT) primers (Roche Molecular Biochemicals) and SuperScript reverse transcriptase (GIBCO/BRL). PCR amplifications were performed with the following primer pairs: AgOr1, f5′-TGGAGTGTTTTGGCTGA-3′ and r5′-TTCCATGCTCTGAAGTACG-3′ [product size: 559-bp cDNA, 922-bp genomic

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: BAC, bacterial artificial chromosome; OR, odorant receptor; AgOR, *Anopheles gambiae* odorant receptor; DOR, *Drosophila melanogaster* odorant receptor; GPCRs, G-protein-coupled receptors; RT, reverse transcription.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database [accession nos. AF364130 (AgOr1), AF364131 (AgOr2), and AF364132 (AgOr3 and AgOr4)].

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DNA]; AgOr2, f5′-CGAACCTCCTTTCTCGTGAT-3′ and r5′-CTTCAGCTCGGCGAACAGTG-3′ (product size: 474-bp cDNA, 597-bp genomic DNA); AgOr3, f5′-GGAAAAGGAGCTGAACGAGA-3′ and r5′-CTAAAACTGCTCCTTCAGTA-3′ (product size: 309-bp cDNA, 367-bp genomic DNA); AgOr4, f5′-ATTTACGGCGGCAGTATCTT-3′ and r5′-TCACTGTACATCCATCTTTA-3′ (product size: 450-bp cDNA, 610-bp genomic DNA); *rps7*, f5′-GGCGATCATCATCTACGTGC-3′ and r5′-GTAGCTGCTGCAAACTTCGG-3′ (product size: 458-bp cDNA, 610-bp genomic DNA); *AgArr1,* f5′-TGGGCAAGCGTGACTTTGTAGA-3′ and r5′-TCTGCTCCTTGTCCGACTTTT-3′ (product size: 231 bp). Optimal annealing temperature was 56°C for AgOR primer pairs and 58°C for *rps7* and *AgArr1*.

Mosquito Rearing and Blood Feeding. *A. gambiae* sensu stricto (G3 strain) embryos were kindly provided by Mark Benedict (Centers for Disease Control and Prevention, Atlanta, GA) or generated in-house and disinfected with 0.05% sodium hypochlorite before hatching in flat plastic pans with distilled water. Larvae were reared on a diet of ground Whiskas Original Recipe cat food (KalKan, Vernon, CA), which was applied to the surface of the water. Pupae were transferred to plastic cups in one-gallon (≈4 liters) plastic containers, where newly emerged adults were collected the next morning. Adult mosquitoes were maintained in one-gallon plastic containers at 27°C with 75% relative humidity under a 12:12 h photoperiod and provided a 10% dextrose solution. Four- to five-day-old female mosquitoes were bloodfed on anesthetized mice for ≈20 min by using standard protocols.

Results and Discussion

In concert with several molecular approaches for the identification of genes expressed in the olfactory system of *A. gambiae*, we have undertaken an informatics-based screen for sequences related to the recently identified family of putative DORs (10–12). With this approach, a modified tBLASTn algorithm (13) was used to screen a partially sequenced collection of *A. gambiae* BAC clones. This analysis identified three BAC clones containing sequences displaying significant similarity to several DORs. More than 12 kb of the three BAC clones were sequenced, revealing four genes, which display homology to DORs and contain multiple transmembrane domains. Accordingly, we have termed these genes candidate *A. gambiae* ORs (AgORs) and have numbered them accordingly: AgOr1, AgOr2, AgOr3, and AgOr4.

An alignment of the deduced amino acid sequences between two AgORs and their most closely related DORs is shown in Fig. 1*A*. In this analysis, AgOr2 has a significant degree of relatedness to Or43a, displaying 36% identity and ≈74% similarity. Or43a is one of the few ORs, and the only DOR, to date that has been functionally characterized (17, 18). Furthermore, the establishment of an odorant profile for Or43a may aid in the functional characterization of AgOr2. AgOr1 has greater than 18% identity and 61% similarity to Or46b. These values are especially persuasive when taken within the context of the high degree of divergence that DORs display among themselves (10, 11).

In addition to primary sequence similarity between AgORs and DORs, an analysis of all four AgORs reveals multiple hydrophobic regions (19) (data not shown), which is characteristic of this family of GPCRs. Moreover, the relative positions of a subset of introns, and the total predicted length of the deduced proteins (averaging 400 aa), are also well conserved among the AgORs and DORs. An alignment of the deduced amino acid sequences of the four AgORs alone (Fig. 1*B*) shows a low degree of overall sequence conservation that has also been characteristic of DOR comparisons (9, 11, 20). The four AgORs share as little as 11% identity and 34% similarity overall, with much of the identity located at the C terminus, where GPCRs in general and ORs in particular are thought to interact with downstream components of the signaling cascades such as GPCR kinases (21, 22) and arrestins (23).

In addition, AgOr3 and AgOr4 are linked tightly together in the *A. gambiae* genome, where they are separated by only 746 bp (Fig. 1*C*). This tendency for close chromosomal linkage is characteristic of many odorant and taste receptor genes from *D. melanogaster* (9–12, 20) and OR genes from *C. elegans* (8) and mouse (24). Furthermore, when comparing AgOr3 and AgOr4, a significant degree of colinearity regarding intron/exon organization, as well as overall sequence conservation, is observed between this pair of AgORs (Fig. 1*D*). In this instance, both the sizes and positions of AgOr3 introns 1 and 3 are highly conserved relative to AgOr4 introns 1 and 2, respectively. If we compare predicted amino acid sequences between these genes, exon 1 displays 23% identity and 36% similarity between AgOr3 and AgOr4, whereas a comparison of the combined exons 2 and 3 from AgOr3 with the larger exon 2 of AgOr4 shows 28% identity and 56% similarity. When comparing AgOr3 exons 4–6 with AgOr4 exon 3, we observe 34% overall identity and more than 60% similarity. These data provide strong arguments that AgOr3 and AgOr4 are derived from a common ancestor by means of a relatively recent duplication event.

To assess their relationships more fully, the four AgOR proteins were aligned with 61 DORs and 32 representative *Drosophila* gustatory receptors (data not shown) to serve as an outgroup, with CLUSTALX v1.8 (14), with minor manual adjustments. From this alignment, phylogenetic trees were generated and bootstrap analysis was used to assess statistical support for these relationships (Fig. 2). In this analysis AgOr1 clusters with DORs 46a and 46b with moderate support, suggesting derivation from a common ancestor gene, whereas AgOr2 clusters confidently with DORs 30a, 43a, and 49b, suggesting they may also represent orthologous genes. In keeping with their chromosomal linkage and suggestive of a recent duplication event, AgOr3 and AgOr4 confidently group together. In contrast, by these analyses, AgOr3 and AgOr4 have no significant orthologs within the family of DORs or *Drosophila* taste receptors. It is intriguing to speculate that AgOr3 and AgOr4 might therefore represent a novel class of mosquito receptors that may be associated with olfactory-driven behaviors that are unique to insects such as *A. gambiae.* These may include, among others, characteristic responses to ovipositional and host preference cues that, in the latter case, for an anthropophilic mosquito such as *A. gambiae,* might largely consist of human-specific odorants.

As an initial step in investigating whether these genes display olfactory-specific expression, RT-PCR was performed. In these studies, 4-day-old adult mosquitoes were dissected into four groups: antennae/maxillary palps (olfactory tissues), head (from which olfactory tissue has been removed, but with proboscis attached), body, and legs. These tissues were used to generate RNA, and subsequently, cDNA pools. All RT-PCRs were performed with oligonucleotide primers that were designed to span predicted introns to distinguish between genomic DNA and cDNA templates, as well as oligonucleotide primers for the *A. gambiae* ribosomal protein S7 gene (*rps7*) (25). The *rps7* gene is constitutively expressed at high levels in all tissues of the mosquito and, therefore, provides a control for the integrity of the cDNA templates.

Olfactory-specific expression of all four AgORs is observed (Fig. 3). RT-PCR products of the predicted size are seen exclusively in reactions with antennae/maxillary palp cDNA templates. No AgOR-specific products are observed with head/proboscis, body, or leg cDNA templates. In all cases, the *rps7* amplifications are more robust for the head, body, and leg templates, reflecting the higher template amounts used in these parallel reactions, further demonstrating there is no detectable expression of AgORs in the nonolfactory tissues examined. In

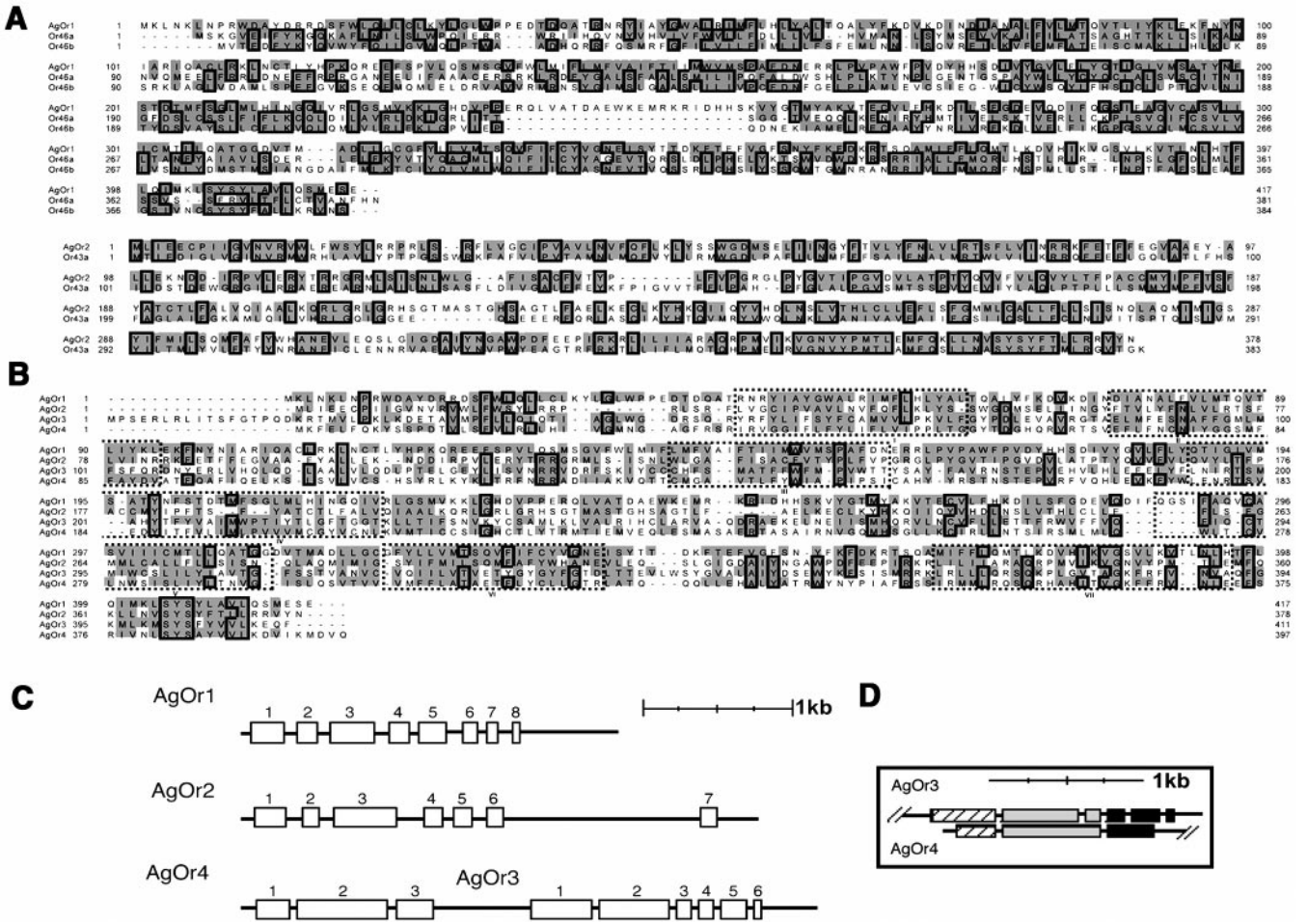

Fig. 1. Deduced amino acid alignments and genomic structure of AgORs. (*A*) CLUSTALX (v1.62b) (14) alignment viewed in SeqVu (The Garvan Institute of Medical Research) of AgOr1 and Or46a and Or46b, its closest related DORs, and AgOr2 and Or43a, its closest related DOR. For all alignments, similarity shading is based on an 85% Goldman–Engelman–Steitz (GES) scale, and identity shading is based on a 65% scale (35) with SeqVu. (*B*) CLUSTALX (v1.62b) (14) alignment viewed in SeqVu of AgORs. Transmembrane domains are indicated by dashed boxes and are numbered below. (*C*) Schematic representation of the intron/exon structure of AgORs and the chromosomal linkage between AgOr3 and AgOr4. The position and relative size of exons and introns are drawn to scale as indicated. (*D*) AgOr3 and AgOr4 intron/exon colinearity. Like-filled boxes represent corresponding exons between deduced amino acid sequences for exon 1 of AgOr3 and AgOr4 (hatched); exons 2 and 3 of AgOr3 and exon 2 of AgOr4 (gray); exons 4–6 of AgOr3 and exon 3 of AgOr4 (black).

several cases, genomic contamination of cDNA templates is detectable and, as a result of primer design, is clearly distinguishable from cDNA products. To verify further the specificity of these RT-PCRs, the RT-PCR products from each AgOR were subcloned and sequenced, revealing in each instance that an AgOR-specific product had indeed been obtained (data not shown). Finally, to ensure that the AgORs are not expressed in any tissues other than antennae/maxillary palp, an additional 15 cycles of PCR were added to the control reactions containing head, body, and leg cDNA templates. Even under these extremely sensitive conditions, AgOR RT-PCR products are undetectable in nonolfactory tissues (data not shown).

We have repeatedly attempted to detect the olfactory-specific expression of the four AgORs reported here by means of *in situ* hybridization but, in each case, obtained inconclusive staining patterns. The inconclusive staining patterns are not entirely surprising, given the low expression levels of DORs, of which a sizable subset (30%) are undetectable by using *in situ* hybridization methods (9). Furthermore, in two studies involving *in situ* hybridization of candidate *Drosophila* taste receptor genes, either none or only a small fraction was detected (20, 26). In light of these studies, it is likely that the expression levels of the four AgOR genes reported here are also beneath the detection threshold for *in situ* hybridization.

Blood feeding and host preference selection are restricted to female mosquitoes and, as such, may be associated with a subset of olfactory genes displaying sex-specific expression. To address this issue we used RT-PCR to examine the sex-specific expression of the AgORs. In these studies (Fig. 4*A*), AgOr1 displays female-specific olfactory tissue expression, whereas AgOr2, -3, and -4 are readily detectable in both male and female olfactory tissue. As in earlier studies, additional PCR cycles were added to ensure that AgOr1 was indeed absent in cDNA prepared from adult male olfactory tissue, whereas amplification of a recently identified *A. gambiae* olfactory arrestin gene *AgArr1* (27) served as a positive control.

Although sex-specific expression of olfactory genes may be indicative of a role in establishing host preference in females, we cannot use these criteria to rule out the importance of any AgOR in this process. Indeed, male mosquitoes from several species have responded to vertebrate host-specific odorants that presumably are associated with mating behavior in the vicinity of the host (28). As noted previously, phylogenetic studies indicate that AgOr3 and AgOr4 do not have close orthologs within the family of DORs (Fig. 2). Therefore, although our data show that AgOr3

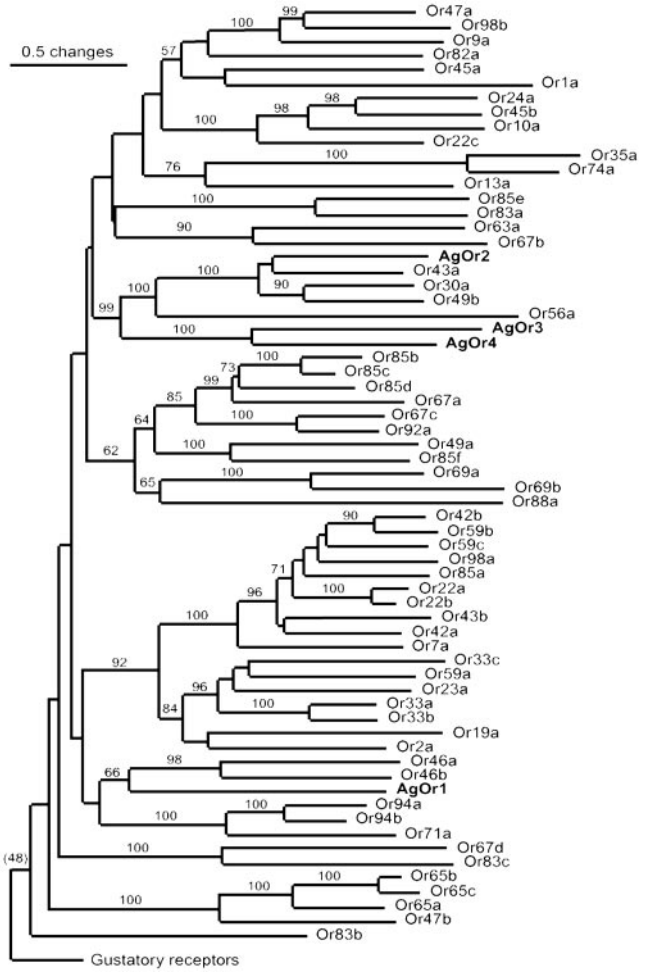

Fig. 2. Phylogenetic analysis of AgORs. Phylogenetic tree showing relationships of the four AgORs (bold type) to those from *D. melanogaster*. The tree was rooted with 32 representatives of the *D. melanogaster* gustatory receptor family. Numbers above branches are the percentage of 1,000 bootstrap replication trees that branch, with only those above 50% shown. The OR family can be readily recognized on several sequence and gene structure features, yet has only 48% bootstrap support, and there is no support for the backbone of the relationships within the family. The scale bar indicates 50% divergence, using distance corrected with maximum likelihood and the BLOSUM62 matrix.

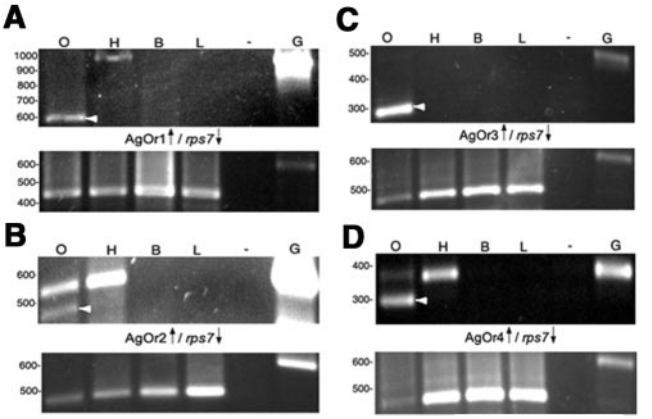

Fig. 3. Olfactory tissue-specific expression of AgORs. Male and female (combined) *A. gambiae* antennae and maxillary palps (O, olfactory tissue), heads stripped of olfactory tissues (H), legs (L), or bodies devoid of appendages (B) were used to generate RNA for RT-PCR. Reaction products, visualized under UV illumination after staining with ethidium bromide, represent the amplification of (*A*) AgOr1 (559 bp), (*B*) AgOr2 (474 bp), (*C*) AgOr3 (286 bp), and (*D*) AgOr4 (309 bp) (indicated by white arrowhead) along with each respective *rps7* control product (458 bp). Higher molecular weight PCR products in AgOr1 (H), AgOr2 (O, H), and AgOr4 (H) represent amplified genomic DNA contamination of RNA samples. A no-template negative (−) control ensures the specificity of the amplicons, and a genomic DNA template (G) reaction indicates the relative position of PCR product derived from genomic DNA contamination in experimental samples. The position of molecular weight markers (bp) is indicated *Left*.

and AgOr4 are expressed in both male and female olfactory tissue, it is nonetheless possible that these genes do contribute to olfactory behaviors unique to hematophagous insects. Further study will be required to establish the precise behavioral pathways on which individual AgORs or, as is more likely the case, groups of synergistically acting AgORs have an impact.

Changes in the responsiveness to host stimuli in response to blood meals have been studied in the yellow fever and dengue vector mosquito *Aedes aegypti* (reviewed in ref. 29). In these studies, significant reductions in olfactory responses have been observed after ingestion of a blood meal, and are postulated to involve mechanisms resulting from the effects of both abdominal distension (30) and the ovarian- and fat body-mediated release of a neuropeptide from the central nervous system (31). Furthermore, electrophysiological recordings have been used to measure changes at the chemosensory level in *A. aegypti,* showing reductions in sensitivity to lactic acid in peripheral olfactory neurons after a blood meal (32). More recently, by using the malaria vector mosquito *A. gambiae,* host-seeking behaviors and electrophysiological responses to human odorants were also shown to decrease significantly in female mosquitoes shortly after ingestion of blood meals (33).

To further address the possibility that AgOr1 might act in olfactory signaling pathways that have an impact on blood feeding and host preference selection in female mosquitoes, we assayed AgOr1 expression in antennae dissected from 4- to 5-day-old female mosquitoes before and 12 h after a blood meal. We observed a dramatic down-regulation of AgOr1 mRNA levels in the olfactory tissue of female mosquitoes 12 h after a blood meal (Fig. 4*B*), whereas levels of *AgArr1* remain constant. Although we cannot exclude a broad down-regulation of several classes of *A. gambiae* genes in response to blood feeding, the fact that mRNA levels of another more generalized component of the olfactory signal transduction cascade, *AgArr1,* remain constant during this interval indicates that this phenomenon is more specific to a subset of olfactory genes. This phenomenon demonstrates that the down-regulation of the expression of a specific gene may, in part, underlie the well-studied phenomenon of decreased host-seeking behavior in disease vector mosquitoes.

A recent example of a similar down-regulation of putative OR expression on odorant stimulation has been shown in *C. elegans,* where low levels of dauer pheromone, a signal of crowding, cause changes including down-regulation in expression of three candidate *C. elegans* chemosensory receptors that are expressed in neurons regulating entry into the dauer stage (34). These results, along with the data presented here, suggest that down-regulation of particular OR expression may provide a means to modify an organism's odorant response profile. Moreover, our data suggest that AgOr1 may act as a component of a specific olfactory signal transduction cascade that is active before blood feeding in *A. gambiae* adult females. As such, this putative OR might be expected to play a critical role in establishing the host preference that is a central element in this mosquito's high overall capacity to transmit malaria.

In this study we have identified and characterized four candidate OR genes, which are homologs of the *D. melanogaster*

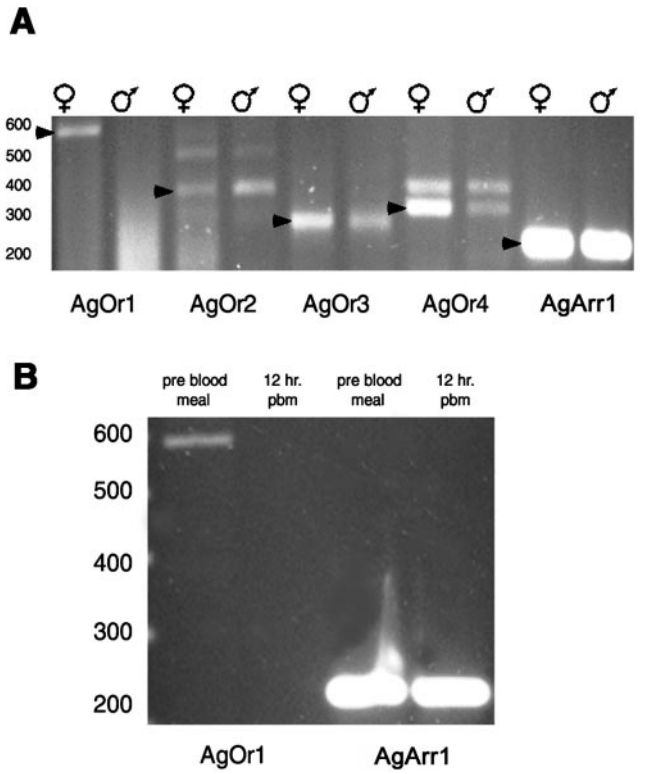

Fig. 4. (*A*) Female-specific expression of AgOr1. Four- to 5-day-old male and female antennae were used to generate RNA for RT-PCR. AgOr1 RT-PCR product (559 bp) is detectable only in female antennae, whereas AgOr2 (474 bp), AgOr3 (286 bp), and AgOr4 (309 bp) are amplified from both male and female antennae. Arrowheads indicate cDNA products; larger bands result from genomic DNA contamination of cDNA templates. An olfactory gene, *AgArr1* (231 bp), is amplified as a control. The position of molecular weight markers (in base pairs) is indicated to the left of the panel. (*B*) Down-regulation of AgOr1 expression after blood meal (pbm). Antennae from 4- to 5-day-old females before blood meal and females 12 h after blood meal were used to generate RNA for RT-PCR. AgOr1 PCR product (559 bp) is detectable only in antennae before blood meal. *AgArr1* (231 bp) is amplified as a control.

ORs, in the malaria vector mosquito *A. gambiae*. To date, we have screened ≈5.3% [14.5 megabases (Mb)] of the *A. gambiae* genome (270 Mb) for the presence of putative AgOR genes, leading to an estimate of ≈80 AgORs in total. This is on the order of current estimates for the number of OR family members in *D. melanogaster* (*Drosophila* Receptor Nomenclature Committee 2000).

With the identification of four members of a family of candidate OR proteins in *A. gambiae,* functional, biochemical, behavioral, and transgenic studies may now be undertaken to determine the specific classes of odorant ligands that activate these receptors, to unequivocally show these genes function as ORs. By focusing on genes such as AgOr1, this process may lend insight into the design of additional compounds that act as mosquito attractants or repellants. Furthermore, comparative studies of putative ORs from hematophagous and nonhematophagous insects, and between anthropophilic and zoophilic species of anopheline mosquitoes, may provide information concerning the molecular basis for host preference selection among these insects. Because of the well-established contribution of olfaction to the vectorial capacity of *A. gambiae*, such insight could conceivably lead to novel disease-prevention strategies for malaria and other historical and newly emerging arthropod vector-borne diseases.

We thank G. Olsen and C. Alexander for the help with the initial tBLASTn searches, C. Warr for assistance in receptor expression experiments and helpful comments on the manuscript, K. Walden for direct sequencing of BAC clones, A. Ray for assistance with amino acid alignments, W. Honegger, C. Merrill, and D. Hartley for comments on the manuscript, P. Russell for mosquito rearing and dissection, and A. McAinsh for editorial assistance. BAC clones were generously provided by F. Collins. This investigation received financial support from the United Nations Development Program/World Bank/World Health Organization Special Program for Research and Training in Tropical Diseases (TDR; V30/181/208 to L.J.Z.) and by grants from the Division of Integrative Biology and Neuroscience of the National Science Foundation (IBN 0075338 to L.J.Z. and IBN 96–04095 to H.M.R.) and the National Institutes of Health/National Institute of Deafness and Other Communication Disorders (1 R01 DC04692–01 to L.J.Z. and R01 DC04729 and R01 DC02174 to J.R.C.).

1. Hildebrand, J. G. & Shepherd, G. M. (1997) *Annu. Rev. Neurosci.* **20,** 595–631.
2. Takken, W. (1991) *Insect Sci. Appl.* **12,** 287–295.
3. Collins, F. H. & Paskewitz, S. M. (1995) *Annu. Rev. Entomol.* **40,** 195–219.
4. Mombaerts, P. (1999) *Annu. Rev. Neurosci.* **22,** 487–509.
5. Pilpel, Y., Sosinsky, A. & Lancet, D. (1998) *Essays Biochem.* **33,** 93–104.
6. Zhao, H., Ivic, L., Otaki, J. M., Hashimoto, M., Mikoshiba, K. & Firestein, S. (1998) *Science* **279,** 237–242.
7. Zhang, Y., Chou, J. H., Bradley, J., Bargmann, C. I. & Zinn, K. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 12162–12167.
8. Troemel, E. R., Chou, J. H., Dwyer, N. D., Colbert, H. A. & Bargmann, C. I. (1995) *Cell* **83,** 207–218.
9. Vosshall, L. B., Wong, A. M. & Axel, R. (2000) *Cell* **102,** 147–159.
10. Clyne, P. J., Warr, C. G., Freeman, M. R., Lessing, D., Kim, J. & Carlson, J. R. (1999) *Neuron* **22,** 327–338.
11. Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A. & Axel, R. (1999) *Cell* **96,** 725–736.
12. Gao, Q. & Chess, A. (1999) *Genomics* **60,** 31–39.
13. Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990) *J. Mol. Biol.* **215,** 403–410.
14. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997) *Nucleic Acids Res.* **24,** 4876–4882.
15. Swofford, D. L. (2001) *PAUP*: Phylogenetic Analysis Using Parsimony (and Other Methods)* (Sinauer, Sunderland, MA), Version 4.0b4.
16. Strimmer, K. & von Haeseler, A. (1996) *Mol. Biol. Evol.* **13,** 964–969.
17. Wetzel, C. H., Behrendt, H., Gisselmann, G., Störtkuhl, K. F., Hovemann, B. & Hatt, H. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 9377–9380.
18. Störtkuhl, K. F. & Kettler, R. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 9381–9385.
19. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
20. Clyne, P. J., Warr, C. G. & Carlson, J. R. (2000) *Science* **287,** 1830–1834.
21. Peppel, K., Boekhoff, I., McDonald, P., Breer, H., Caron, M. G. & Lefkowitz, R. J. (1997) *J. Biol. Chem.* **272,** 25425–25428.
22. Boekhoff, I., Michel, W. C., Breer, H. & Ache, B. W. (1994) *J. Neurosci.* **14,** 3304–3309.
23. Kieselbach, T., Irrgang, K. D. & Ruppell, H. (1994) *Eur. J. Biochem.* **226,** 87–97.
24. Xie, S. Y., Feinstein, P. & Mombaerts, P. (2000) *Mamm. Genome* **11,** 1070–1078.
25. Salazar, C. E., Mills-Hamm, D., Kumar, V. & Collins, F. H. (1993) *Nucleic Acids Res.* **21,** 4147.
26. Scott, K., Brady, J., R., Cravchick, A., Morozov, P., Rzhetsky, A., Zuker, C. & Axel, R. (2001) *Cell* **104,** 661–673.
27. Merrill, C. E., Riesgo-Escovar, J., Pitts, R. J., Kafatos, F. C., Carlson, J. R. & Zwiebel, L. J. (2001) *Proc. Natl. Acad. Sci. USA*, in press.
28. Takken, W. & Knols, B. G. J. (1999) *Annu. Rev. Entomol.* **44,** 131–157.
29. Klowden, M. J. (1996) in *Olfaction in Mosquito-Host Interactions*, eds. Bock, G. R. & Cardew, G. (Wiley, New York), pp. 212–225.
30. Klowden, M. J. & Lea, A. O. (1979) *J. Insect Physiol.* **25,** 583–585.
31. Brown, M. R., Klowden, M. J., Crim, J. W., Young, L., Shrouder, L. A. & Lea, A. O. (1994) *J. Insect Physiol.* **40,** 399–406.
32. Davis, E. E. (1984) *J. Insect Physiol.* **30,** 179–183.
33. Takken, W., van Loon, J. J. A. & Adam, W. (2001) *J. Insect Physiol.* **47,** 303–310.
34. Peckol, E. L., Troemel, E. R. & Bargmann, C. I. (2001) *Proc. Natl. Acad. Sci. USA* **98,** 11032–11038.
35. Riek, R. P., Handschumacher, M. D., Sung, S. S., Tan, M., M. J., G., Schluchter, M. D., Novotny, J. & Graham, R. M. (1995) *J. Theor. Biol.* **172,** 245–258.