NEUROPHYSIOLOGY

A sensory system for mating in octopus

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Sensory systems for mate recognition maintain species boundaries and influence diversification. Thus, uncovering how molecules and receptors evolve to mediate this critical function is essential to understanding biodiversity. Male octopuses use a specialized arm called the hectocotylus to identify females and navigate their internal organs to reach the oviduct and deliver sperm. Here, we discovered that the hectocotylus is a dual sensory and mating organ that uses contact-dependent chemosensation of progesterone, a conserved ovarian hormone. We identified chemotactile receptors for progesterone and resolved the structural basis for their evolution from ancestral neurotransmitter receptors and subsequent expansion and tuning across cephalopods. These findings reveal principles by which sensory innovations shape reproductive behavior and suggest mechanisms for how sensory evolution contributes to the diversification of life.

Sensation is a gateway for reproduction across life by mediating the recognition of potential mates. Therefore, sensory receptors are evolutionary hotspots that can preserve conspecific recognition or drive interspecies mating, which underlies biodiversity (*1*–*3*). Thus, understanding how sensory receptors evolve and are tuned to recognize mating partners is critical to understanding the origins of new species.

Octopuses are solitary animals that mate upon infrequent encounters (*4*). Males use a specialized arm called the hectocotylus to identify females. The hectocotylus navigates toward and within the female's mantle, distinguishing the oviducts from other organs to deposit a spermatophore that moves the length of the arm from the male mantle to the hectocotylus tip. Relatively few studies, which are largely based on anecdotical evidence, describe octopus mating, leaving important questions unanswered (*5*–*11*). How does the male recognize a correct mate during such rare encounters? How does the hectocotylus navigate the internal female organs to inseminate the oviducts?

Octopuses use their arms for "taste-by-touch" chemotactile sensation of the seafloor, raising the possibility that mate recognition is associated with contact-dependent sensation (*12*). Octopus predation is driven by a complex, distributed arm nervous system (*13*–*16*) with chemotactile receptors (CRs) that diverged from ancestral neurotransmitter receptors, which use structural adaptations to facilitate contact-dependent chemosensation of hydrophobic compounds (*17*, *18*). Although CRs have been shown to detect poorly soluble metabolites secreted by surface microbiomes of prey (*19*), it is unknown how octopuses detect mates.

Here, we report that octopus mating is driven by contact-dependent chemosensation of the conserved ovarian hormone progesterone. Our study describes the hectocotylus as a sensory organ for mating and highlights how evolution can sculpt sensory receptors for mate detection, which is essential for the diversification of species.

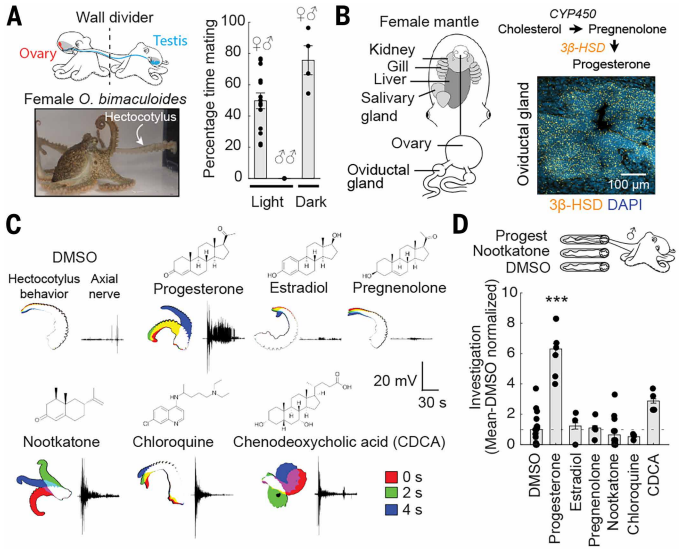

Fig. 1. Octopuses use sex steroids to guide chemosensory behavior. (**A**) *O. bimaculoides* mated through a barrier with openings for the hectocotylus (left). Shown on the right is the percentage of time spent mating (defined as when the male hectocotylus reaches inside the female mantle) quantified in both light conditions (n = 3 mating pairs, 49.7 ± 5.1%) and dark conditions (n = 2 couples, 75.7 ± 9.3%). Male-male couples did not exhibit mating behavior (n = 3 couples). (**B**) The location of the ovary and oviducts in which sperm cells are deposited by the hectocotylus during mating (left) and expression of the essential enzyme for progesterone biosynthesis 3β-HSD) (yellow) in the oviductal gland visualized by in situ hybridization (right). Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; blue). Images are representative of n = 3 animals. (**C**) Axial nerve activity and autonomous behavior from amputated octopus hectocotyluses in response to steroids, bile acids, and bitter molecules. n = 3 to 6 animals; $P < 0.005$ for responses to 100 μM progesterone, as determined by one-way analysis of variance (ANOVA) with Kruskal-Wallis test. Summary data are provided in fig. S1F. (**D**) In mating behavioral trials, females were removed from the barrier tank and replaced with tubes in the openings for the hectocotylus. Shown are the times spent by male octopuses investigating tubes coated with progesterone, structurally similar steroids, bile acids, terpenes, or vehicle (n = 4 to 15 trials; data are normalized to vehicle; $P <$ 0.001 for progesterone, as determined by one-way ANOVA with Kruskal-Wallis test). In (A) and (D), error bars are mean ± SEM. DMSO, dimethyl sulfoxide.

Octopus mating is driven by female chemosensory cues

To determine which sensory cues are required for octopus mating, we first separated two octopuses (wild-caught *Octopus bimaculoides*) by an opaque barrier with small openings through which they could introduce their arms in a controlled environment (Fig. 1A). To our surprise, even with minimal visual information, the male extended the hectocotylus through the barrier and carefully maneuvered toward and subsequently inserted the specialized appendage within the female mantle. After insertion, the hectocotylus extended deep within the mantle and eventually stopped, at which point both the male and female paused all movement, sometimes for more than an hour during spermatophores transfer (fig. S1, A and B, and movie

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S1). Similar results were obtained across numerous male-female pairs, even in the absence of visible light, but not when using male-male couples (Fig. 1A). Male-male couples interacted by touching their arms, but they did not attempt mating, suggesting the existence of a female-specific cue (Fig. 1A).

We next reasoned that a putative female-specific cue could originate from female-specific reproductive organs, namely the oviducts and ovary. Comparative tissue-based transcriptomics and gene ontology analysis showed an enrichment of biosynthetic enzymes important for the production of sex steroids and other known bioactive lipid molecules in the ovary and skin sample. This is consistent with observed steroid production during octopus mating season (*20*, *21*) and conserved roles in animal reproduction and development (*22*, *23*) (fig. S1C). In situ hybridization confirmed that oviducts expressed mRNA encoding the biosynthetic enzyme 3β-hydroxysteroid dehydrogenase (3β-HSD), which is responsible for the conversion of the precursor pregnenolone into progesterone (Fig. 1B). Consistent with the expression of steroid biosynthetic enzymes, we also detected progesterone in the ovary, oviduct, and skin of wild-caught females (table S1).

Considering that we observed markers for steroid biosynthesis in the oviducts, which are targeted by the hectocotylus during mating, we wondered whether the hectocotylus also detects sex steroids to guide mating behavior. We exploited the fact that severed arms exhibit neural activity and behavior to find that exogenously applied progesterone evoked robust hectocotylus activity (Fig. 1C). By contrast, structurally similar hormones such as estradiol or the precursor pregnenolone failed to elicit such behavior (Fig. 1C). Indeed, the robustness of the response of the hectocotylus to progesterone was comparable to that observed for nonsexual arm signals such as terpenes, bitter molecules, bile acids, and touch (fig. S1, D to F). These results demonstrate that progesterone elicits a selective response that is locally detected and processed to trigger hectocotylus behavior.

To determine whether progesterone is sufficient to drive mating, we turned back to whole-animal behavior with the same paradigm that we had used to define the chemosensory drive for mating. Similar to our initial experiments, the male and female were separated by a barrier, and the male searched for the female. Next, before mating took place, the female was replaced by conical tubes that were coated with chemical stimuli and attached to the small holes in the barrier in which the male inserted the hectocotylus to initiate mating. Tubes coated with progesterone were sufficient to elicit mating search behavior, with the male actively exploring the progesterone tube in place of searching the female mantle (Fig. 1D and movie S2). This contrasted with tubes coated in other steroid derivatives, bile acids, terpenes, or bitter molecules, which were previously shown to elicit aversive behavior in predation and exploration by nonmating arms (Fig. 1D). Thus, we concluded that progesterone is a robust signal for octopus mating.

The hectocotylus is a conserved sensory and mating organ

Whereas nonmating arms are used for chemotactile exploration and predation, the hectocotylus is almost exclusively used for mating and often even protected during hunting (*4*, *24*). During copulation, sperm packages are transferred from the testis to the base of the hectocotylus and slid down the arm through an external skin groove to the arm tip for release inside the oviducts (*4*) (Fig. 2A). Is the hectocotylus also a specialized sensory organ? A clue that this might be the case came from scanning electron microscopy, which revealed

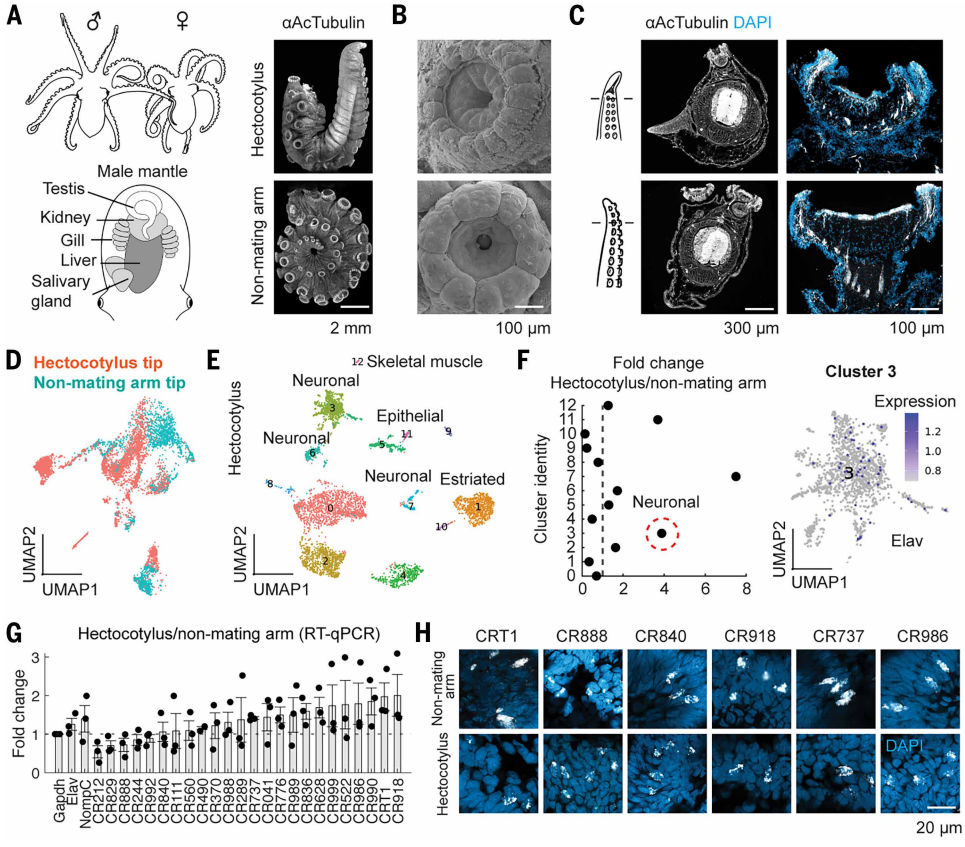

Fig. 2. The hectocotylus is a chemosensory and mating organ. (**A**) Spermatophores are generated in the mantle, transported down the hectocotylus arm to the tip, and deposited at the oviduct openings in the female mantle (left). Shown on the right are tissue-cleared arm tips stained with the neuronal marker acetylated α-tubulin. (**B**) Scanning electron micrographs of sucker cups in the hectocotylus tip and nonmating male arm. (**C**) Cross sections of the hectocotylus (top) and nonmating arm (bottom) immunostained against α-acetylated tubulin (white). DAPI nuclear staining was used to visualize the overall tissue morphology. Images are representative of $n = 3$ animals. (**D**) Uniform manifold approximation and projection (UMAP) reduction of snRNA-seq data from sucker sensory epithelium in the hectocotylus tip (4266 nuclei) versus the nonmating arm tip (2702 nuclei) (see also fig. S2). (**E**) A UMAP of clustered hectocotylus cells shows multiple neuronal clusters that were identified by the expression of the neuronal marker gene Elav in at least 10% of cells. (**F**) The largest neuronal cluster (cluster 3) represents 25% of all hectocotylus tip cells (1067 of 4266) versus 6.4% in the nonmating arm (174 of 2702). The vertical dashed line indicates no change; the red dashed circle highlights a neuronal cluster. (**G**) CR expression measured by quantitative RT-PCR and normalized to the housekeeping gene glyceraldehyde phosphate dehydrogenase (Gapdh). Data were analyzed as hectocotylus divided by nonmating arm. The horizontal dashed line indicates equal expression in the hectocotylus and nonmating arm. Error bars are mean ± SEM. $n = 3$ animals. (**H**) CR expression in the hectocotylus tip and nonmating arm sensory epithelium visualized by in situ hybridization (white staining). Nuclei were stained with DAPI (blue). Images are representative of $n = 6$ animals.

that the hectocotylus tip was covered by small sucker cups, similar to those found in regular arms and used in chemotactile sensation of the seafloor and prey (Fig. 2B). Furthermore, tissue clearing and immunohistochemical staining revealed dense neuronal innervation of the hectocotylus sucker cups, comparable to that of a nonmating arm tip (Fig. 2C). These anatomical observations are consistent with a direct sensory role for the hectocotylus, as suggested by the physiology and behavior of isolated arms (Fig. 1C and fig. S1, D to G).

To determine whether the hectocotylus exhibited molecular and cellular features indicative of a sensory organ, we used comparative single-nucleus RNA sequencing (snRNA-seq) to profile the hectocotylus tip that contacts the ovaries versus the base arm, which is similar to the other nonspecialized arms used for predation. After profiling ~13,000 nuclei across arms, we classified cellular clusters based on enriched transcripts and found that the base arms contained similar cellular populations as defined by a combination of cell types and differential expression (fig. S2A). By contrast, profiling revealed three distinct populations that were expanded in the hectocotylus versus nonmating arm tips (Fig. 2, D to F). Two hectocotylus-enriched clusters were related to metabolism and secretion, and the third (cluster 3) was neuronal and enriched for the gene ontology term "response to chemical stimulus" (fig. S2, B to G). Although sensory receptors are often expressed at low abundance, even snRNA-seq showed that cluster 3 contained CRs, the mechanoreceptor NOMPC, and the neuronal marker Elav, collectively suggesting a sensory function (fig. S2C). Quantitative reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization confirmed that CRs are robustly expressed in the epithelium of the small suckers found along the hectocotylus tip (Fig. 2, G and H). Thus, the hectocotylus exhibits all the molecular and cellular hallmarks of a multimodal sensory organ for chemosensation and mechanosensation, consistent with our physiological and behavioral data.

Because sensory systems for mating are central to evolutionary fitness, we hypothesized that key anatomical and molecular components would be present across cephalopods. To test this possibility, we compared the putative sensory systems for mating in *O. bimaculoides* with those of phylogenetically and geographically diverse octopods (*O. rubescens* and *Abdopus aculeatus*) and a decapod (*Euprymna berryi*) (Fig. 3A). First, we found that ovaries from all cephalopods expressed conserved biosynthetic enzymes for producing sex steroids, including progesterone (fig. S3A), consistent with previous studies in *O. vulgaris* (*20*, *22*). In the hectocotylus, we observed previously described species-specific differences in the distal calamus and ligula but found that all species contained similar sucker cups (Fig. 3B) and robustly responded to exogenously applied progesterone and other sex steroids to a varying extent across species (Fig. 3C and fig. S3B) (*4*, *8*). Responses to progesterone were comparable to responses to CR ligands sensed by nonmating arms, such as terpenes, bitter molecules, and bile acids (Fig. 3C

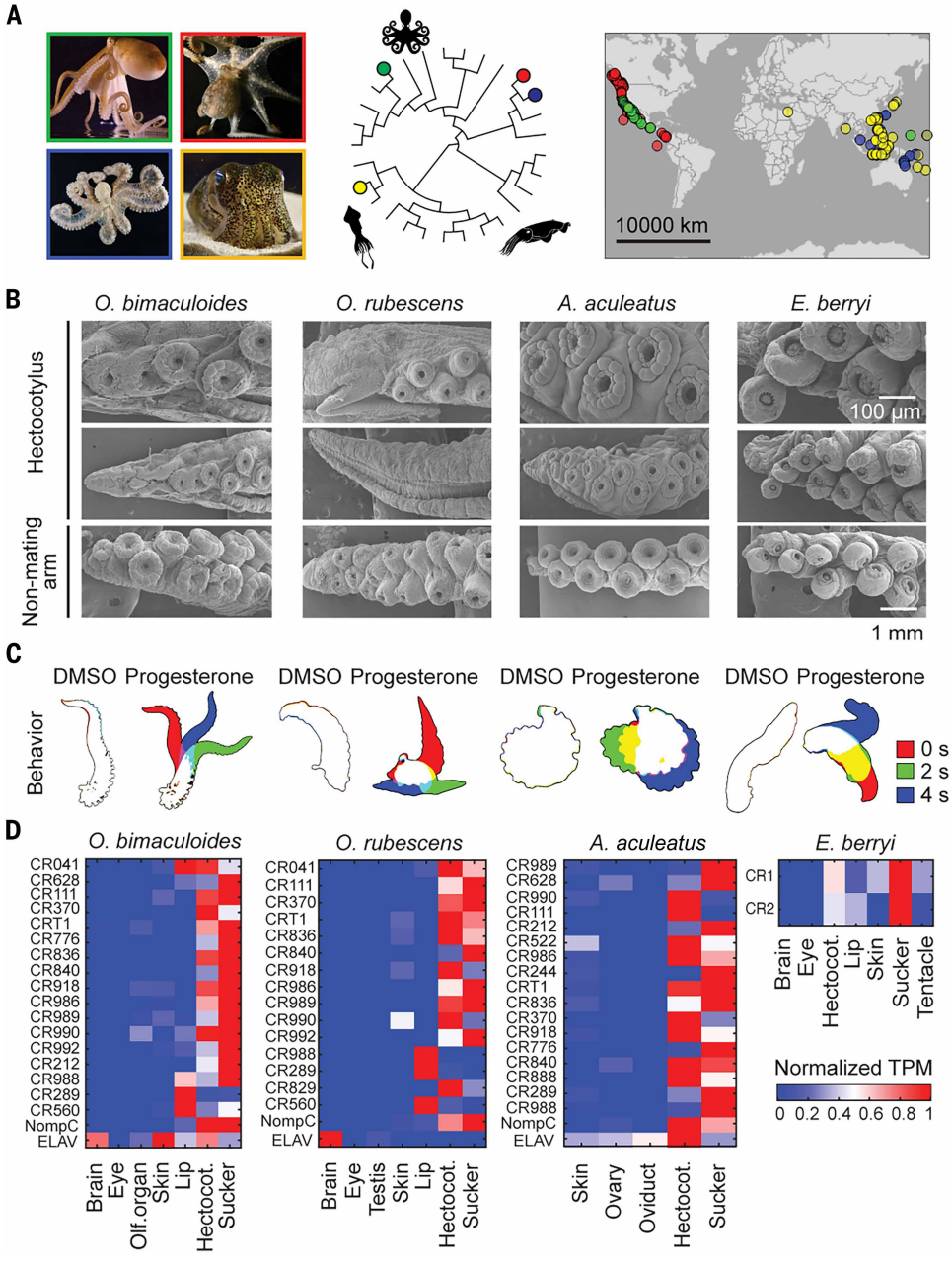

Fig. 3. Steroid sensing is conserved among cephalopods. (**A**) Octopus and squid species analyzed for behavior and transcriptomics with their phylogeny and geographic distribution: *O. bimaculoides* (green), *O. rubescens* (red), *A. aculeatus* (blue), and *E. berryi* (yellow). (**B**) Scanning electron micrographs of hectocotylus and nonmating arms from *O. bimaculoides*, *O. rubescens*, *A. aculeatus*, and *E. berryi*. Images are representative of $n = 4$ animals. (**C**) Autonomous hectocotylus behavior in response to progesterone versus vehicle from *O. bimaculoides*, $n = 3$; *O. rubescens*, $n = 4$; *A. aculeatus*, $n = 8$; and *E. berryi*, $n = 7$. $P < 0.004$ for progesterone, one-way ANOVA with Kruskal-Wallis test. See summary data for other steroids, bile acids, and terpenoids in fig. S3B. (**D**) CR expression in the hectocotylus of *O. bimaculoides*, *O. rubescens*, *A. aculeatus*, and *E. berryi* compared with that in other tissues. The color scale shows transcripts per million (TPM) normalized across samples. Hectocot., hectocotylus.

and fig. S3B). The molecular markers for sensory function were conserved, as CRs were enriched in the hectocotylus tips of all species (Fig. 3D and fig. S3C). Finally, consistent with these findings, the phylogenetically distant *A. aculeatus* also mated in the absence of any visual cue (fig. S3D), suggesting that chemosensory-driven mating is not limited to *O. bimaculoides*.

CRT1 is a rapidly evolving progesterone receptor in octopus

We next sought to identify the progesterone receptor of the hectocotylus. We started by screening a panel of 800 bioactive lipids, including steroids, across five different CRs that were most abundantly expressed in the hectocotylus and could be functionally probed in expression systems (fig. S4, A to C, and table S2). We found that only one CR, CRT1, robustly responded to sex steroids, including progesterone (Fig. 4, A and B, and table S3). Patch-clamp electrophysiology with CRT1 demonstrated that progesterone is the highest-affinity natural ligand for an octopus CR reported to date [Fig. 4C; (*19*)]. This response was also selective because CRT1 was insensitive to the structurally similar precursor pregnenolone, matching the pharmacological profile of hectocotylus neural activity and behavior (Fig. 4C). Indeed, a cryo–electron microscopy (cryo-EM) structural analysis of CRT1 bound to progesterone demonstrated that progesterone was coordinated within the canonical orthosteric binding site (Fig. 4, D and E, and fig. S5A). The density for progesterone was strong but ambiguous as to which orientation the steroid was bound in the pocket. We used molecular dynamics simulations to identify the most energetically favorable orientation of progesterone in the cryo-EM density map (fig. S5, B and C). Substitution of residues coordinating progesterone markedly reduced progesterone-evoked activity (Fig. 4F), supporting the inference that this solvent-exposed site is the route through which the lipidic agonist acts.

CRT1 was previously characterized as a receptor for metabolites produced by the microbiota on the surface of prey (Fig. 5A) (*19*). How does the same receptor mediate both steroid recognition for mating and metabolite sensing for predation? By comparing the structures of CRT1 bound to norharmane (predation) or progesterone (mating), we found that both had an open-pore conformation and a similar permeation pathway, consistent with their ability to activate CRT1 (fig. S6). Yet compared with norharmane, progesterone had an increased interaction area and inferred binding energy with three residues of the orthosteric binding site, which are Leu^{153} (L153), Leu^{191} (L191), and Tyr^{39} (Y39) (Fig. 5, B and E, and fig. S7A).

The conserved residue L153 forms the back wall of the conserved orthosteric pocket, coordinating the acetyl chain on the D-ring of progesterone (Fig. 5B and fig. S7B). We reasoned that if this conserved region provides a general groove for hydrophobic ligands, then altering its hydrophobicity should similarly affect binding of both progesterone and norharmane. Indeed, substituting L153 for a hydrophobic alanine residue increased binding affinity for both ligands, whereas the hydrophilic residues (glutamine and asparagine) decreased affinity (Fig. 5, C and D, and fig. S8, A to E). Additionally, replacing other conserved residues within the hydrophobic groove reduced binding of both ligands (fig. S7D). These results suggest a conserved site for coordinating poorly soluble ligands that is important in contact-dependent aquatic sensation of predatory metabolites or steroids in mating.

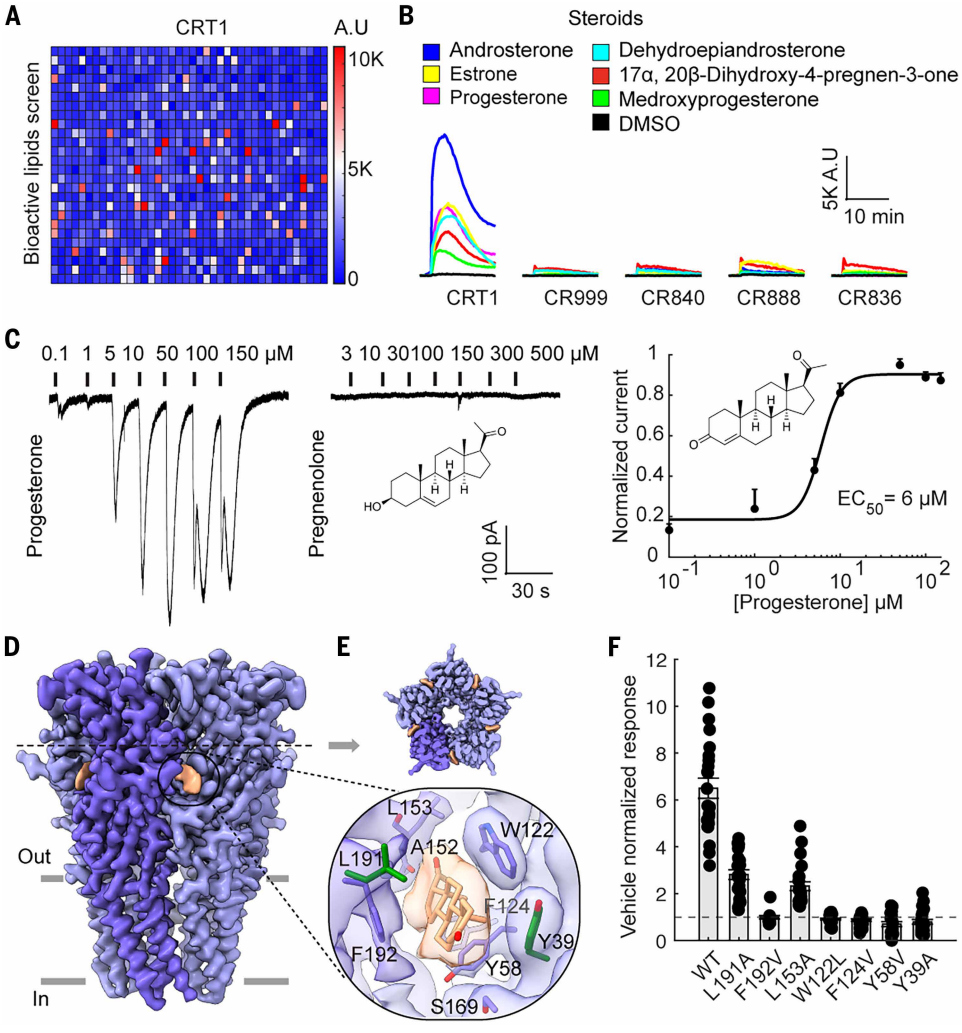

Fig. 4. CRT1 is a steroid receptor with high affinity for progesterone. (**A**) *O. bimaculoides* CRT1 activity in response to a panel of 800 bioactive lipid molecules (10 μM). Responses were quantified by the increase in fluorescence of GCaMP6-expressing cells as an indicator of CR activity over basal. Each cell represents the top response to a single molecule. The color bar indicates fluorescence in arbitrary units (A.U.). (**B**) Comparison of the sensitivity of all tested CRs to sex steroids. Traces represent raw responses from single trials shown in (A). (**C**) Electrophysiological measurements from cells expressing CRT1 in response to progesterone and its precursor pregnenolone [$n = 6$ cells, median effective concentration (EC_{50}) = 5.8 ± 0.5 μM for progesterone]. High concentrations produced a reduction of the peak amplitude and fast desensitization consistent with channel-pore block. (**D**) Cryo-EM density map of octopus CRT1 bound to progesterone. Progesterone is highlighted in orange. (**E**) Shown at the top is the top view of this cross section in the cryo-EM map. Shown at the bottom is the map and atomic model of the orthosteric ligand binding site, showing coordination of the most favorable progesterone pose. Interacting residues are shown as sticks. (**F**) Responses from CRT1 with point mutations in residues that coordinate progesterone. Responses are normalized to vehicle. $n = 8$ to 24 replicates; $P < 0.001$ for wild type (WT) versus mutants in response to 15 μM progesterone, as determined by one-way ANOVA with Kruskal-Wallis test. In (C) and (F), error bars are mean ± SEM. A, Ala; F, Phe; S, Ser; V, Val; W, Trp.

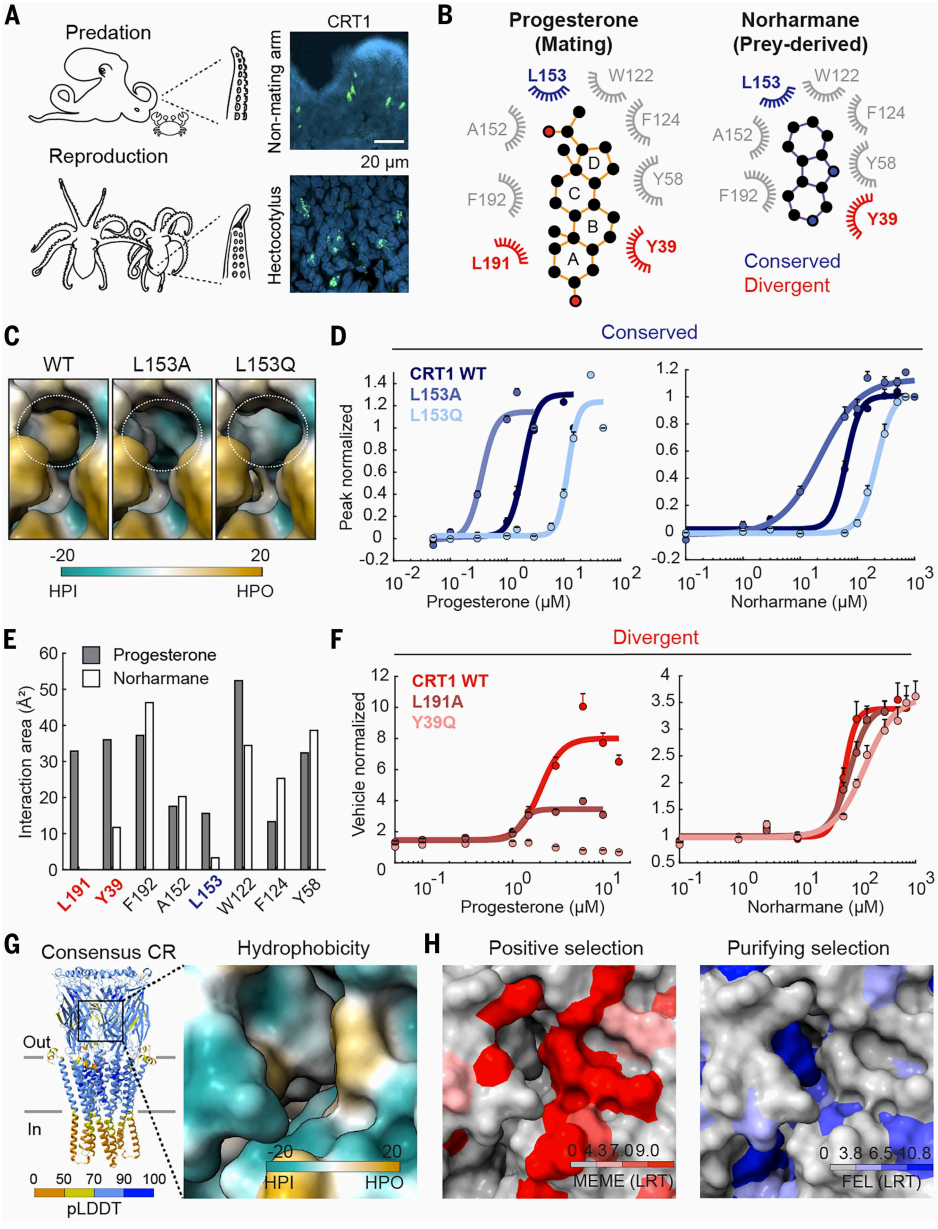

Fig. 5. Structural adaptations of CRT1 underlie high specificity to steroids. (**A**) CRT1 expression in the hectocotylus and nonmating arm of octopuses visualized by in situ hybridization (green staining). Nuclei were stained with DAPI (blue). Data are representative of $n = 8$ experiments. (**B**) Binding pocket residues coordinating progesterone (left) and norharmane (right) highlighting the conserved residue L153 colored in blue and divergent residues L191 and Y39 colored in red. Structural and selection analyses in fig. S7. (**C**) Binding pocket hydrophobicity and volume comparison in CRT1 with Leu^{153}→Ala (L153A) and Leu^{153}→Gln (L153Q) substitutions. Dashed white circles indicate the L153 position. (**D**) Progesterone and norharmane dose-response activity relationships in CRT1 with L153A and L153Q mutations. Progesterone: EC_{50} WT = 2.2 ± 0.2 μM, $n = 24$ replicates; L153A = 0.4 ± 0.0 μM, $n = 24$; L153Q = 12.3 ± 0.5 μM, $n = 16$. Norharmane: EC_{50} WT = 75.6 ± 6.6 μM, $n =$ 32 replicates; L153A = 19.8 ± 2.0 μM, $n = 24$; L153Q = 247.7 ± 19.4 μM, $n = 16$. (**E**) Interaction area for residues L191, Y39, and L153 with progesterone and norharmane. (**F**) Progesterone and norharmane dose-response activity relationships in CRT1 with the L191A and Y39Q mutations. Mean progesterone peak response (15 μM, vehicle normalized): WT = 6.5 ± 0.4, $n = 24$ replicates; L191A = 2.8 ± 0.2, $n = 24$; Y39Q = no response, $n = 16$. Norharmane response (150 μM, vehicle normalized): WT = 2.6 ± 0.2, $n = 28$ replicates; L191A = 2.8 ± 0.2, $n = 16$; Y39Q = 2.0 ± 0.2, $n = 12$. (**G**) Predicted AlphaFold3 (*28*) structure of a consensus CR obtained from 235 CR sequences across 29 cephalopods, including octopus, squid, and cuttlefish species. Shown on the right is the hydrophobicity of the predicted ligand binding pocket in a cephalopod consensus CR. HPI, hydrophilicity; HPO, hydrophobicity; pLDDT, predicted local distance difference test. (**H**) Residues in the binding pocket colored by the LRT value for the positive-selection pressure test MEME (left) and purifying selection test FEL (right). In (D) and (F), error bars are mean ± SEM.

By contrast, L191 and Y39 formed a distinct hydrophobic sandwich around the A-ring at the bottom of progesterone with an increased interaction area compared with that of norharmane, which extended beyond the canonical binding pocket for norharmane (Fig. 5, B and E). These residues were under diversifying selection, indicating that they are rapidly evolving, unlike the other residues in the conserved pocket that coordinates both molecules (fig. S7C). Consistent with these observations, mutating L191 and Y39 showed a stronger effect on progesterone sensitivity than on norharmane sensitivity (Fig. 5F and fig. S8, A to E). Collectively, these results suggest that CRT1 has functionally diversified in two ways: (i) CRT1 is expressed in sensory suckers of both the nonmating arm and mating hectocotylus, and (ii) it has evolved from a hydrophobic binding pocket that loosely binds molecules important for predation to a high-affinity steroid binding pocket for molecules involved in mating (Fig. 5A).

Although the hectocotylus from all tested cephalopods was sensitive to progesterone, we noticed varying sensitivities to related steroid derivates (fig. S3B). As such, we wondered whether CRs are broadly diversifying structural regions that could tune steroid binding to support species-specific chemosensory behaviors, including recognizing mating partners. To answer this question, we used genomic sequences from 30 cephalopod species (235 total receptors) to build a consensus receptor and used AlphaFold3 to predict its structure. The consensus orthosteric site resembled the relatively flat hydrophobic pocket of CRT1, indicating a common biophysical property of CRs that allows binding of relatively insoluble molecules (Fig. 5G). We then overlaid the likelihood ratio test (LRT) value for positive [mixed effects model of evolution (MEME)] and purifying selection [fixed effects likelihood (FEL)] to determine which regions are under evolutionary pressure (Fig. 5H). These analyses revealed conservation of the inner pocket—the same region that is important for both progesterone and norharmane sensitivity in CRT1—and diversifying selection at the outer region that mediates high affinity for progesterone. This contrasted with ancestral nicotinic acetylcholine receptors, which exhibited generally conserved binding sites compared with those of more recently derived CRs (fig. S7, E to G). These results suggest that CRs evolved a core binding motif suited for taste-by-touch chemotactile sensation of poorly soluble molecules in aquatic environments. This function underwent diversification, enabling distinct behaviors ranging from predation to mating by evolution of the outer binding pocket.

Discussion

Chemoreceptor families expand and contract to meet lineage-specific ecological and behavioral demands. Similar to vomeronasal and variant ionotropic receptors in other animals, CRs evolved from an ancient neurotransmitter receptor scaffold. In contrast to animals that evolved distinct receptor families to support diverse behaviors, octopuses use a single family for predation, maternal behavior, and mating. In a wide range of animals, chemosensitive and reproductive organs remain separate: Pheromones are detected by dedicated sensory receptors and epithelia, such as in the rodent vomeronasal organ (*25*), whereas mating organs act as effectors. The octopus hectocotylus breaks this modularity by collapsing chemosensation and sperm transfer into a single appendage, similar to the male reproductive organ of some insects (*26*, *27*). This integrated function ensures mate assessment with respect to sex, age, seasonality, and likely numerous other factors that modulate progesterone amounts and reception to mating. This all occurs at the site and moment of gamete delivery, a strategy well suited to the rare encounters of these solitary animals.

Cephalopods represent a rich source of biological innovation. Along with abandoning the protection afforded by the ancestral molluscan shell, these animals evolved long-distance locomotion, active predation, and problem solving. This shift coincided with the evolution of flexible arms, an advanced distributed nervous system, and CRs as a sensory-receptor expansion that underpins their ecological success. Among the most profound departures from their molluscan relatives is reproduction. Whereas bivalves and gastropods rely on hermaphroditism and broadcast external spawning, cephalopods evolved separate sexes, sex chromosomes, and direct transfer of spermatophores. The evolution of sexual reproduction between distinct male and female individuals imposes selective pressure on precise mate recognition and timing. Thus, by merging precise sperm transfer and sensory innovations suited to contact-dependent chemosensation, the cephalopod hectocotylus may have reinforced species-specific sensory boundaries, contributing to reproductive isolation and facilitating diversification.

Sensory innovation and reproduction are repeatedly entwined because animals rely on complementary sensory modalities and cues to find mates. Insects use refined pheromone receptors for species recognition, mammals diversify sensory repertoires of receptors involved in mate choice, and cephalopods remodel arms into combined chemosensory-reproductive organs. In each lineage, evolution has reshaped conserved receptor scaffolds to serve reproductive systems, and cephalopods push this integration further by fusing sensation and sperm transfer into a single appendage. This fusion not only secures reproductive efficiency but also may have generated new pathways that contribute to lineage diversification. Indeed, hectocotylus CRs exhibit high sequence divergence among orthologous genes, which may support functional differences in steroid sensing to support species recognition and subsequent mate selection. Thus, our investigation of octopus mating serendipitously identified sensory receptors as molecular hotspots for interrogating reproductive isolation and speciation.

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SUPPLEMENTARY MATERIALS

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Materials and Methods; Figs. S1 to S8; Tables S1 to S8; References (*28*–*48*); Reproducibility Checklist; Movies S1 and S2

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A sensory system for mating in octopus

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Editor's summary

Male octopuses use a specialized arm called the hectocotylus to navigate inside of the female mantle and toward the ovary to deposit spermatophores for fertilization. The mechanisms determining the success of this strategy have remained unknown. Villar *et al*. have now demonstrated that progesterone produced in female ovaries activates hectocotylus neural activity and autonomous movement and stimulates male mating search behavior (see the Perspective by Di Cosmo). Sensory cells in the hectocotylus expressing the receptor CRT1 are responsible for sensing progesterone. These results describe the molecular basis of a previously unrecognized sensory organ for mating in octopus and shed light on how sensory innovation determines reproductive success. —Mattia Maroso

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