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Essay

Paraneurons

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The evolution of neurons has captured the imagination of generations of neuroscientists, developmental biologists and evolutionary biologists alike. In 1975, Tsuneo Fujita introduced the term ‘paraneurons’ to name a wide array of cell types within and outside the central nervous system that show no overall morphological similarity to neurons but use a prominent and easily visible secretory apparatus to secrete neuropeptides in response to specific stimuli. Fifty years later, we now understand that most paraneurons are either exteroceptors or interoceptors, signaling to neurons or endocrine effector cells of the nervous system. Moreover, we now appreciate that many, and perhaps all, paraneurons are genetically specified by a class of transcription factors that are broadly involved in inducing neuronal fate, the proneural basic helix-loop-helix transcription factors. Viewed through the lens of neuronal cell type evolution, paraneurons may provide a unifying framework for the evolution of neurons, with the key determining feature being a common genetic program that induces sensory-secretory characters in extant neurons, paraneurons and perhaps ancient ‘proto-neurons’.

Even before Santiago Ramon y Cajal’s comprehensive microscopical explorations of many cell types constituting animal brains, neurons had been appreciated as cells with long, thin processes, termed ‘axons’ and ‘dendrites’ by Kölliker and His, respectively[1]. These specific morphological elaborations allowed Cajal to postulate the principle of neuronal polarity and Sherrington to describe a synapse as the contact point between neuronal processes[1]. The enormous diversity in shape and form of axodendritic processes of neurons prompted Cajal to poetically label neurons as the ‘butterflies of the soul’[2].

As the cellular composition of the central and peripheral nervous system was mapped out in more detail over the ensuing decades after Cajal’s pioneering work, it became clear that other cells that were in more or less close proximity to neurons also displayed a pronounced secretory apparatus but did not show the anatomical elaborations (i.e. axons or dendrites) that conventional neurons usually display (Figure 1). In 1975, Tsuneo Fujita proposed to subsume these cells under the term ‘paraneurons’, using the Greek prefix ‘para’ for ‘beside’ or ‘next to’[3]. Even though Fujita elaborated on the description of these cells in several publications, including a full-scale book[4], the term has not found wide usage. I revisit here the paraneuron concept and summarize what we have learned about these cells over the past few decades, both in regard to their function and also shared developmental specification programs. In the spirit of Dobzhansky’s dictum “nothing makes sense in biology except in the light of evolution”[5], I suggest that it may be useful to consider paraneurons from an evolutionary perspective. Paraneurons may be reflective of a recurrent ‘proneural’ process that employs a shared regulatory program (‘module’) to generate neuron-like cells. The co-option of this module may have happened not only early in the evolution of neuronal cell types, but multiple times in functionally diverse epithelial cells of derived tissue types in more complex organisms as complex organ types arose later in evolution.

Typology of paraneurons

As per their original definition, all paraneurons are receptosensory in nature and display a prominent secretory apparatus[3,4] (Figure 1). Paraneurons, as defined by Fujita, fall into two broad superclasses: sensory-type paraneurons that signal via afferent fibers toward the peripheral or central nervous system, and effector-type paraneurons that receive neuronal input to control physiological processes via hormonal secretions (Figure 2).

Sensory-type paraneurons

These types of paraneurons fall into two categories, exteroceptive and interoceptive paraneurons. Exteroceptive paraneurons are mechanosensory hair cells and mechanosensory Merkel cells, as well as taste cells. None of these cells have traditionally been considered as neurons but are excitable cells that relay sensory stimuli to primary afferent nerve fibers. In contrast to exteroceptive paraneurons, which perceive signals from the environment, interoceptive paraneurons sense distinct internal sensory modalities in response to which they signal either to afferent neurons or to the bloodstream. Interoceptive processes have received significant attention over the past few years, but this recent literature has regrettably not used the term ‘paraneurons’[6]. Like exteroceptive paraneurons, interoceptive paraneurons are embedded in epithelial surfaces within the body. These vertebrate cells include (Figure 1): gastroenteropancreatic neuroendocrine cells along the alimentary tract that produce and secrete hormones in response to cues derived from ingested food; carotid body chief cells (aka glomus cells) lining the circulatory system, which measure O_2, CO_2, pH and metabolites in arterial blood to then signal directly to afferent fibers of the carotid sinus nerve; bronchopulmonary neuroendocrine cells lining the respiratory system, which are polymodal sensors for inhaled O_2 and CO_2 levels, volatile odors and mechanical stimuli that signal to afferent fibers of the vagus nerve; urogenital paraneurons lining the urethra and other parts of the urogenital tract, which perceive mechanical and chemical stimuli and relay this sensory information to afferent fibers that innervate these paraneurons; parafollicular cells of the thyroid (also known as C cells), as well as parathyroid chief cells, both epithelial cells that are stimulated by high calcium levels in the blood to regulate calcium homeostasis via the release of parathyroid hormone. Although traditionally not considered as interoceptors, adenohypophysal endocrine cells, another set of cells called paraneurons by Fujita (Figure 1), should perhaps also be considered interoceptive, since they are epithelial cells that respond to signals in the blood, namely, hormones released by the hypothalamus.

Effector-type paraneurons

Fujita also classified several heavily innervated endocrine gland cells as paraneurons, namely adrenal chromaffin cells and pinealocytes (Figure 1). Adrenal chromaffin cells release catecholamines in response to stressor signals that are conveyed via sympathetic nerve fibers innervating these gland cells. Pinealocytes, the main cell type in the pineal gland (considered the 'seat of the soul' by René Descartes[7]), release melatonin in response to signals relayed by sympathetic nerve fibers. In early-branching vertebrates, pinealocytes are photosensitive, which make these cells also exteroceptors[8]. Much like muscle cells, glandular paraneurons that receive direct innervation can be considered 'end organs' or 'effector cells' of the nervous system since they translate signals received from the nervous system to an organismal output (Figure 2). What makes a gland cell a paraneuron is that it has a specialized sensory apparatus, with the 'sensory' part being an efferent fiber that innervates the gland cell to exert the regulated secretion of peptides.

The above typology of paraneurons shows that the term is not entirely synonymous with neuroendocrine cells. While all neuroendocrine cells are paraneurons, exteroceptive paraneurons, like hair or taste cells, are not considered to be neuroendocrine cells. The same can be said about another traditional (and also little used) cell type classification system, the APUD series of neuroendocrine cells, named after their ability of '<u>A</u>mine <u>P</u>recursor <u>U</u>ptake and <u>D</u>ecarboxylation'[9], which encompass most, but not all, neuroendocrine cells but again not exteroceptive paraneurons[4]. Taken together, the paraneuron term is a broad, yet clearly delineated umbrella term for cells with functional and organizational similarities, unifying sensory receptors located on external or internal epithelial cells feeding into the nervous system, as well as effector cells of the nervous system that respond to neuronal stimuli by releasing hormonal cues that control various physiological processes (Figure 2).

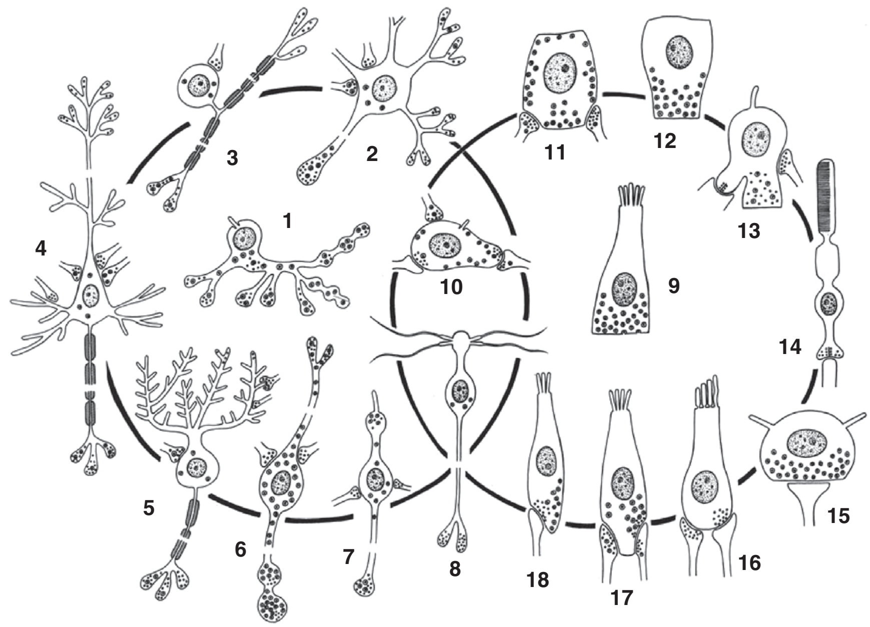

Figure 1. Neurons and paraneurons.
The spectrum of neurons (left) and paraneurons (right), as drawn by Fujita *et al.*[4] 1: enteric neuron, which is considered by Fujita as the prototype of neurons; 2: multipolar autonomic neuron; 3: sensory neuron; 4: pyramidal neuron; 5: Purkinje cell; 6: 'neurosecretory' or 'peptidergic' neuron; 7: CSF-contacting neuron; 8: olfactory cell; 9: gut endocrine cell, which for Fujita represents the prototype of paraneurons; 10: carotid body chief cell; 11: adrenal chromaffin cell; 12: adenohypophysial, parafollicular, or other endocrine cell; 13: pinealocyte; 14: visual cell; 15: Merkel cell; 16: inner ear hair cell; 17: bronchopulmonary paraneuron; 18: gustatory cell. (Reproduced from Fujita *et al.*[4] with permission by Springer Nature.)

While the discussion up to this point has primarily concerned vertebrates, paraneurons also abound in invertebrates. For example, the nematode *Caenorhabditis elegans,* whose entire anatomy has been delineated in exquisite detail, contains cells that can be classified as paraneurons, including sensory-type interoceptors (g1 and g2 glands in their foregut and interoceptive uv1 paraneurons in their uterus) as well as innervated effector paraneurons, the excretory gland cells[10]. *Drosophila melanogaster*, too, possesses enteroendocrine cells that fulfill paraneuronal criteria[11]. Several glandular cells in more basal metazoans, like cnidarians, could also be considered paraneurons, particularly those that share lineage relationships with conventional neurons[12].

Developmental specification of paraneurons

Another remarkable unifying feature of paraneurons has emerged with the rise of molecular genetics and concerns their apparently shared developmental specification program. Many — and perhaps all — paraneurons are genetically specified by a specific, sequence-related subgroup of basic helix-loop-helix (bHLH) transcription factors oftentimes referred to as 'proneural' genes[13–15]. Proneural genes were identified in the 1990s through genetic studies showing that loss of individual family members leads to either a failure of neuroectodermal cells to adopt a neuronal fate (hence, 'proneural bHLH') and/or a failure of neuronal precursors to terminally differentiate[13,14]. Vice versa, ectopic expression of these proneural factors suffices to impose neuronal features onto normally non-neuronal cells[13,14]. This function appears to be conserved across animal phyla and to coincide with the evolution of the nervous system. For example, in two different cnidarian species, characterized by comparatively simple nerve net structures, proneural bHLH factors appear to control neurogenesis[16,17]. In one of them, a homolog of the

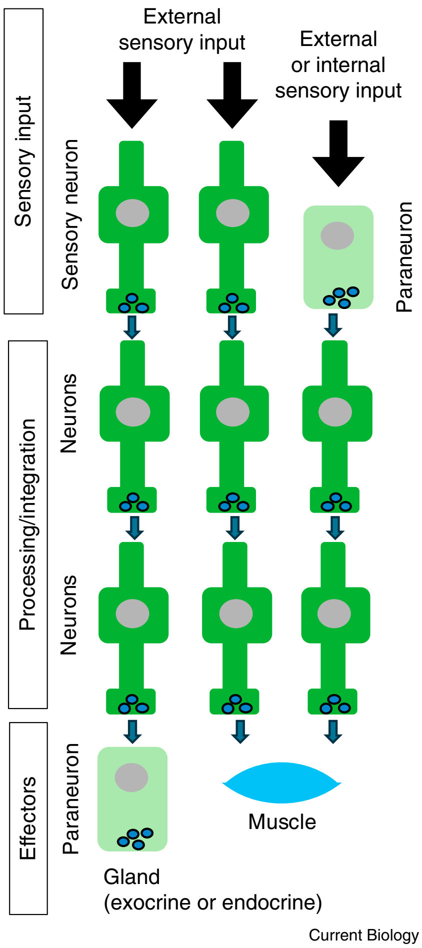

Figure 2. Extant paraneurons are integrated into the nervous system.
Paraneurons serve as extero- or interoceptors that provide information to the nervous system or they serve as effector cells of the nervous system akin to the other major effector cell type of the nervous system, muscle.

bHLH factor Achaete-Scute (ASc) also selectively marks a digestive gland cell[18], a potential paraneuron. Ctenophores, whose nervous system has been proposed to have evolved independently[19], do not have genes encoding bHLH proteins that can clearly be assigned to the proneural, Atonal (Ato) or ASc subgroups[20,21], but this issue needs to be re-evaluated once more ctenophore genomes become available.

The expression and function of bHLH proteins has been examined in many paraneurons and, remarkably, in all cases examined Ato/ASc-like bHLH genes have been found to be required for their developmental specification. Exteroceptive paraneurons such as Merkel cells or hair cells require the proneuronal Atoh1 bHLH transcription factor for their specification[22,23]. Similarly, interoceptive paraneurons also require proneuronal bHLH proteins for their proper development. Specifically, Mash1 is required for the proper specification of gastric neuroendocrine cells, carotid body chief cells, bronchopulmonary paraneurons and parafollicular cells of the thyroid[24–27]. Mutations in human Neurogenin3 reduce the number of intestinal enteroendocrine cells, resulting in congenital malabsorptive diarrhea[28]. Adenohypophyseal paraneurons also require proneural bHLHs for their proper specification[29], as do cells in the third category of paraneurons, glandular effector cells, as exemplified by the Mash1-dependence of adrenal chromaffin cells[30]. Some paraneurons, such as urogenital paraneurons, have not yet been examined for their dependence on proneuronal bHLH factors but, given ample precedent from other paraneurons, their involvement is to be expected as well.

The function of bHLH factors in specifying paraneurons is not restricted to vertebrates. In *Drosophila*, enteroendocrine cells, the likely homologs of vertebrate enteroendocrine paraneurons, also require proneural bHLHs for their proper specification[31,32]. Similarly, in *C. elegans*, the pharyngeal gland cells, also paradigmatic examples of paraneurons, require an ASc homolog, *hlh-6*, for their proper differentiation[33].

Paraneurons from the perspective of the evolution of neurons

It has been long speculated that neurons evolved from a primitive sensory-neurosecretory ‘proto-neuron’ in a division-of-labor process that made some cells become committed to receiving and relaying signals and others specialized to generate movement[12,34–36]. The sensory-neurosecretory nature of such proto-neurons, initially perhaps devoid of neurites and synapses, is conceptually akin to extant extero- and interoceptor paraneurons. Another step in the evolution of neurons is the generally presumed delamination of such sensory-neurosecretory proto-neurons from an epithelial cell layer, followed by the elaboration of neurites and specialization of sensory and secretory apparatuses into synaptic structures. Extant effector-type paraneurons may again be reflective of such a process, with sensory-neurosecretory cells having indeed delaminated, receiving inputs from, say, sensory-neurosecretory proto-neurons, but still using their neuropeptidergic secretory apparatus, without elaborating any neurites.

Hence, one may be tempted to argue that extant paraneurons are ‘living fossils’ that have not (yet) taken the next step in progression to a more neuron-like state (Figure 3A). However, since many extant paraneurons in vertebrates exist in tissue types that are clearly relatively recent inventions (e.g. bronchopulmonary paraneurons in airways), it is perhaps more sensible to think about paraneurons as having evolved independently several times in different tissue types as they become more sophisticated in perceiving and relaying sensory information. In other words, the progression of an epithelial cell to a sensory-neurosecretory cell may not have been a singular event that happened deep in the evolutionary past at the advent of what we recognize as nervous systems today but has rather happened again and again during animal evolution, following a similar trajectory (Figure 3B).

The implication of proneural bHLHs in specifying paraneurons provides a mechanistic basis for the recurrent generation of paraneurons. Evolutionary novelties are now generally recognized to be driven by specific regulatory modules that have been coopted into novel cellular contexts[37–39]. In the context of paraneurons, the module would be a bHLH to sensory-secretory regulatory module, with the bHLH transcription factors controlling the expression of genes that code for receptors and secretory machinery. This module may have become coopted multiple times, independently in distinct epithelial contexts to endow epithelial cells with the ability to express sensory/secretory features.

The evolutionary ancestry of bHLH-dependent sensory-neurosecretory modules is illustrated in basal, non-bilaterian organisms that lack a nervous system. Sponges have no neurons but contain secretory globule cells, suggested to be sensory in nature and these cells express a group A bHLH gene, AmqbHLH1[40]. Remarkably, AmqbHLH1 has proneural activity when expressed in *Drosophila* or even *Xenopus*[40].

Another, quite distinct hypothesis for the evolutionary history of paraneurons is that they may represent a regressed, 'degenerate' state of a neuron, or, in other words paraneurons may be secondarily simplified (Figure 3C). Indeed, it has been argued that vertebrate mechanosensory cells, specifically hair cells, may have shared a common ancestor with the more clearly neuron-like mechanosensory receptor cells of invertebrates[41]. Such a simplification may be the result of a loss of a bHLH-dependent regulatory submodule involved in neurite formation and growth. Since genetic screens in worms and flies have not revealed single gene candidates for such a loss, such secondary simplifications would need to be multistep processes and would have to occur independently multiple times if they were indeed a common basis for the evolution of paraneurons. Irrespective of whether or how often such a secondary simplification origin scenario may apply, the deep evolutionary connection between extant paraneurons and neurons would not be questioned by such a scenario.

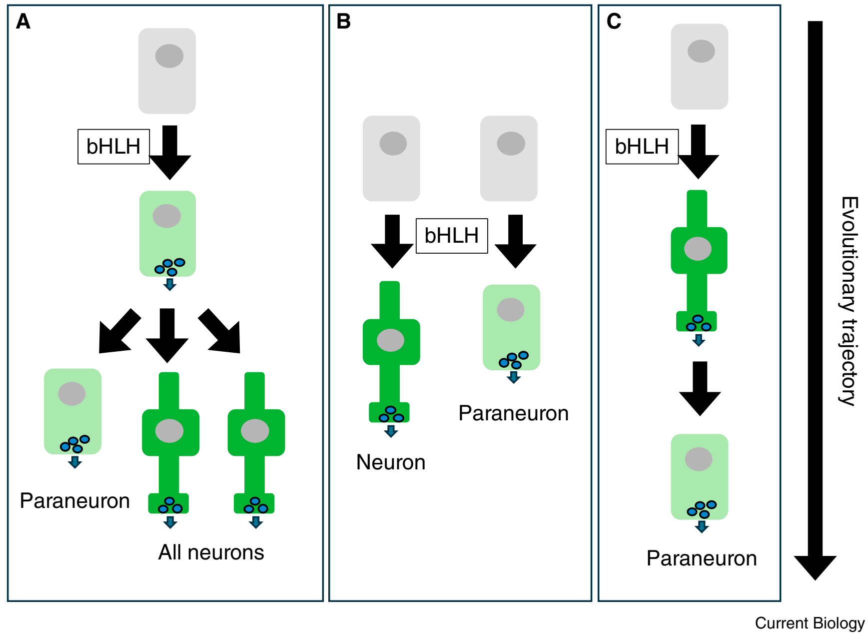

Figure 3. Hypothetical scenarios for the evolutionary trajectories of paraneurons.
(A) Paraneurons may be evolutionary precursors to neurons that originated from epithelial precursors (grey) via a bHLH regulatory module imposing secretory features (blue secretory vesicles). Extant paraneurons may not have adapted more neuron-like properties. (B) Paraneurons and neurons may have arisen independently but using the same regulatory modules (proneural bHLH) for their respective origins. (C) Paraneurons may be secondarily derived from neurons through a process of simplification. Note that these scenarios are not mutually exclusive; different types of paraneurons may have taken any one of these trajectories.

Open questions

How do bHLH transcription factors drive the expression of paraneuronal features? The simplest possibility is that bHLH proteins directly bind to *cis*-regulatory elements in genes coding for sensory and secretory proteins to initiate and properly maintain their expression. This is perhaps the case in the most basal metazoans, such as the secretory cells of sponges, or in sensory cells of cnidarians. However, in bilaterians, the expression of bHLH transcription factors, both in neurons and in paraneurons is transient, i.e. is downregulated in fully mature, adult cells (with some notable exceptions, discussed below)[14,15,42]. Since sensory/secretory genes are continuously expressed throughout the life of these paraneurons, other factors must have taken on the role of ensuring their maintained expression. Moreover, within the nervous system, extensive analysis of the regulatory logic of pan-neuronal, secretory gene modules in *C. elegans* has failed to provide evidence for direct regulation of these genes by bHLH transcription factors[43,44]. Hence, bHLH transcription factors may operate via intermediary factors to control neuronal/paraneuronal features. In many neurons, transiently acting proneural bHLH factors operate via master-regulatory terminal selectors to initiate and maintain neuronal cell type identity (e.g., see Masoudi *et al*.[42]). Many such terminal selectors are homeodomain transcription factors[45]. It will be interesting to see whether a similar terminal selector-based logic exists in paraneurons as well and whether distinct paraneurons are made by distinct combination of homeodomain transcription factors acting downstream or perhaps even in cooperation with bHLH proteins.

Intriguingly, there are at least two cases, one neuronal and one paraneuronal, in which the expression of an Ato/ASc-type bHLH is maintained and in both cases the bHLH factor cooperates with homeodomain proteins. In vertebrate Merkel cells, a prime example of a paraneuron, Atoh1 is continuously expressed during terminal differentiation and cooperates with the LIM homeodomain transcription factor Islet1 to maintain the differentiated state of Merkel cells[46]. Such maintained expression is also observed in a neuronal case in *C. elegans*, the ADL sensory neurons, which required continuously expressed *hlh-4* and LIM homeobox gene *lin-11* to initiate and maintain the differentiated state[47]. The possibility of an ancestral bHLH/homeobox partnership in driving neuron-like states will require more detailed expression and functional studies.

The case of the *Drosophila* Ato family member Dimmed provides an auxiliary perspective to the function of bHLH proteins. Dimmed is expressed in highly secretory cells, including peptidergic neurons, and has been proposed to act as a 'scaling factor' to boost the expression of neuropeptides and their secretory apparatus[48]. The very broad expression of Dimmed insinuates that other factors — such as terminal selector-type homeodomain transcription factors — control which neuropeptides or specific signaling molecules are selectively expressed in a given cell type and Dimmed then boosts their expression further. Such a scaling role of Dimmed could either be a convergent aspect of bHLH function or again be a reflection of an ancestral ground state in which the original secretory phenotype was driven by bHLH factors alone and then diversified by the addition of cell type-specific terminal selectors.

From an evolutionary standpoint, the association of bHLH factors with sensory-neurosecretory cells in basal metazoans, i.e. early branching non-bilaterians, clearly requires more attention. While a function for bHLH proteins in sensory cells of sponges has been inferred[40], the expression and function of bHLHs needs to be analyzed in greater depth in placozoans (which have no nervous system but do contain specialized secretory cells) and ctenophores (which contain neurons that may have evolved independently of those in other animals). An intriguing recent transcriptomic analysis defined several types of peptidergic cells in placozoans, a subset of which express proneural Ato/ASc-type bHLH transcription factors[49]. Whether this subset of peptidergic cells is more neuron-like in terms of a sensory function and regulated release of peptidergic signals will require further analysis.

Concluding remarks

The usefulness of the paraneuron terminology lies in bringing together a seemingly diverse group of extant cells, located in different parts of the body, ranging from outer and inner epithelial sensory cells, to neuroendocrine cells, to gland cells. The unifying properties of these cells are specialized receptor-secretory features and the usage of a shared genetic specification program. Paraneurons may constitute prime examples for the concept of gene regulatory modules as important drivers of phenotypic evolution, perhaps shedding light on evolutionary processes that have generated animal nervous systems.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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Socially parasitic ant queens chemically induce queen-matricide in host workers

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Matricide — the killing of a mother by her own genetic offspring — is rarely observed in nature, but not unheard-of. Among animal species in which offspring remain with their mothers, the benefits gained from maternal care are so substantial[1] that eliminating the mother almost never pays, making matricide vastly rarer than infanticide. Here, we report matricidal behavior in two ant species, *Lasius flavus* and *Lasius japonicus*, where workers kill resident queens (their mothers) after the latter have been sprayed with abdominal fluid by parasitic ant queens of the ants *Lasius orientalis* and *Lasius umbratus.*

The few known cases of matricide are confined to terrestrial invertebrates and fall into two categories: voluntary matriphagy, where the mother offers her own body as food to her nymphs (found in a species of earwig, a pseudoscorpion and several spider species; Supplemental information), and worker-driven matricide in eusocial hymenopterans, such as bumblebees and vespine wasps, where producing males can benefit workers more than supporting a queen[2]. In ants, queens are also eliminated in non-parasitic contexts — for example, when queen number is regulated in pleometrotic (temporarily multi-queen colony founding) associations or during the formation of hierarchies in functionally monogynous species, where aggression among queens can precipitate worker attacks[3]. No clear example exists in which neither mother nor offspring profits from matricide.

We observed this form of matricide in temporarily socially parasitic ants: newly mated queens of *L. orientalis* and *L. umbratus* (Hymenoptera: Formicidae) invade congeneric host colonies of *L. flavus* and *L. japonicus*, covertly approach the resident queen (Figure 1A and Video S1), and spray multiple jets of abdominal fluid at her (Figure 1C,E,F and Video S1). The fluid appears to be formic acid and is delivered from the acidopore, both unique adaptations of Formicinae[3] (including the genus *Lasius*). This spraying elicits abrupt attacks by host workers (Figure 1B,G and Video S1), which ultimately kill their own mother (Figure 1D,G and Video S1). The parasitic queens are then accepted, receive care from the orphaned host workers and produce their own brood to found a new colony. Our findings are the first to document a novel host manipulation that prompts offspring to kill an otherwise indispensable mother.

Temporary social parasitism — one of the parasitic life-history strategies in ants — faces a single, unavoidable hurdle: the invading queen must eliminate the resident queen, which is fiercely protected by her workers. Yet, despite this perilous requirement, this strategy has evolved independently on multiple occasions across the Formicidae, indicating repeated evolutionary gains of the capacity for queen-killing[4]. Until now, the only mechanistically documented solution was direct assault: the parasite throttles or beheads the host queen, a tactic that has arisen convergently in several lineages[4]. Historical observations in *Lasius* already noted host–worker matricide following parasite-queen introduction — *L. niger* workers killing their queen after the introduction of a *L. umbratus* queen[5] — although the parasite's inducing behaviours were not described. Our study uncovers the basis of this second route — behavioural manipulation — in which the parasite covertly provokes host workers to kill their own mother. This discovery expands the evolutionary repertoire available for the origin of temporary social parasitism. Although we did not test a parasite–host queen pair in isolation, the immediate retreat of the parasite queen after spraying, the prolonged latency to host-queen death, and the escalation of worker aggression (Video S1) indicate that worker attacks are essential to the lethal outcome.

Intriguingly, *L. orientalis* and *L. umbratus* sit in distantly related

