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Evolution

Dicyemids are higher animals

Dicyemids, which are microscopic parasites of squids and octopuses, have among the simplest body plans of all multicellular animals. They lack body cavities and almost all the organs that characterize animals, such as a gut or nervous system, and their development proceeds without germ layers and gastrulation. The adult body consists of a solitary axial cell surrounded by a single layer of 10–40 ciliated outer cells. Here we use information from Hox gene sequences to investigate the phylogenetic affinities of dicyemids, and conclude that dicyemids are lophotrochozoans that have secondarily lost many morphological characters, so the simplicity of their body plan is not a primitive condition.

Because of their simple body plan, dicyemids have long been the subject of phylogenetic controversy. They comprise most of the Mesozoa, a name that indicates a level of complexity intermediate between protists and Metazoa[1]. Alternatively, they have been suggested to be derived from parasitic platyhelminths[2] (dicyemids are obligate symbionts found in the renal sacs of cephalopod molluscs) or placed within the protists[3]. Data from 18S-ribosomal DNA indicate that dicyemids may be related to triploblasts (bilaterians), although these analyses do not resolve whether dicyemids are primitive or degenerate triploblasts[4]. Dicyemids are recognized as primitive animals, although their phylogenetic placement relative to other basal taxa such as Porifera, Placozoa and Cnidaria is unclear.

We isolated genomic and complementary DNA clones for a Hox gene, *DoxC*, from the dicyemid mesozoan *Dicyema orientale*. We used high-stringency Southern-blot analysis and the polymerase chain reaction (PCR) with *DoxC*-specific primers to confirm that *DoxC* is derived from the dicyemid genome: both methods detected the gene in dicyemid DNA but not in host squid DNA (data not shown). The homeodomain sequence indicates that *DoxC* is a member of the 'middle' group[5] of Hox (or Hox-like) genes, and identity is highest with *Antp* and its orthologues. The middle group of Hox genes has only been reported from triploblasts; no cnidarian genes fall into this group[6].

A diagnostic peptide motif is encoded immediately carboxy-terminal to the homeodomain (Fig. 1). This 'spiralian peptide'[7] (also called the Lox5 peptide[8]) has only been reported from the Antp orthologue in lophotrochozoans[9], a group that includes annelids, ribbon worms, brachiopods and planarians (recently assigned to this clade). The peptide motif is not present in any other Hox proteins, including the Antp orthologue of ecdysozoan protostomes (which include nematodes, arthropods and priapulids), the Ubx/abd-A-type proteins of ecdysozoans or lophotrochozoans, or any proteins from deuterostomes or cnidarians. The presence of the spiralian peptide indicates that *DoxC* is the dicyemid orthologue of *Lox5* from leeches, polychaetes and brachiopods, *LsHox6* from ribbon worms and *Dthox-C*, *Dthox-E* and *Pnox-7* from planaria. It also implies that dicyemid mesozoa are not basal and primitive animals and should not be excluded from Metazoa.

Rather, our data argue that dicyemids are members of the Lophotrochozoa and are related to phyla such as platyhelminths, molluscs, nemerteans, brachiopods and annelids. We conclude that dicyemids are secondarily simplified from higher protostome animals and that their body plan is enormously reduced as a result of their endoparasitic lifestyle. Along with the enigmatic Myxozoa, they represent one of the most extreme cases of secondary reduction of body-plan complexity.

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Figure 1 Comparison of deduced amino-acid sequences of the homeodomain (underlined) and carboxy-terminal flanking region for Hox proteins related to Antp. The dicyemid Hox gene, *DoxC*, encodes a spiralian peptide motif assigning it to the Lophotrochozoa. Sequences shown for comparison derive from a platyhelminth (Dthox-C and Dthox-E), polychaete (CTs-Lox5), leech (Hr-Lox5), ribbon worm (LsHox6), nematode (mab5), Antp (fruitfly), sea urchin (TgHox1), cephalochordate (AmphiHox6) and mouse. The *DoxC* sequence has been deposited in the GenBank database, accession number AB030175. See Supplementary Information for the sequences studied and their accession numbers.

Insect behaviour

Male beetles attracted by females mounting

Intrasexual mounting is performed by males and females of many taxa[1], and female–female mounting occurs in insects, lizards, birds and mammals[1,2]. Although the adoption by females of other male-like characters, such as mimicry of male colour patterns[3–5], is known to be advantageous, the benefits of female–female mounting have remained mysterious. Here we describe a pattern of female–female mounting in the beetle *Diaprepes abbreviatus* (Curculionidae) and demonstrate that it conveys a possible evolutionary advantage by providing a greater opportunity for the females to mate with larger males. This explanation may also apply to female intrasexual mounting in several other insect species.

We collected adult weevils from their food plants in Homestead, Florida, and observed them mounting females (Fig. 1). Female and male mounting was similar, and two females could easily be mistaken for a
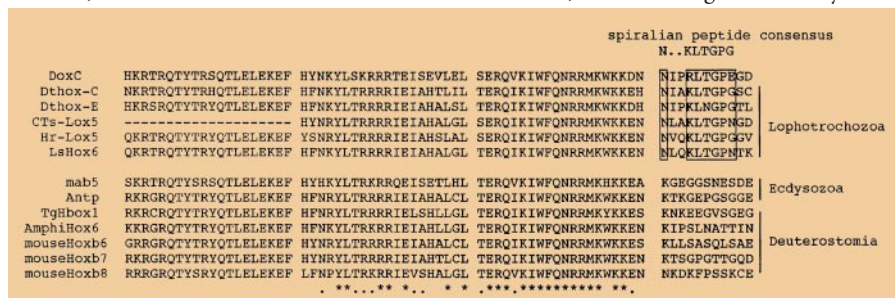
Figure 1 Female-female mounting in *Diaprepes abbreviatus*. The mounting female positions herself along the mounted female's back and extends her ovipositor as she does during oviposition, so it touches the lower posterior part of the mounted female.

pair *in copula*. Females showed no obvious response to mounting and remained together for up to 17.0 min (mean, 9.6 min) in the laboratory, compared with more than 10 hours when males mounted females[6]. Pairing ended either when the female dismounted (17 of 44 cases) or when a male contacted a female (27 of 44), at which point he was equally likely to mate with either female (13 males mated the mounting female, 11 mated the mounted female, and three males mated neither).

D. abbreviatus males and females are monomorphic, but males are slightly smaller than females (50% overlap in elytron length: male 8.7 ± 1.1 mm, female 10.3 ± 1.2 mm; *t*-test, d.f. = 812, $P<0.001$). Males are attracted by volatile chemicals and pheromones but have difficulty discriminating aggregating females[6], and often mount other males or a copulating male–female pair. In the absence of reliable cues, males search either for larger individuals, as these are more likely to be females, or for mating couples, as one individual is likely to be female. When a male approaches a mating couple, he pushes the mating male off the female's back, and larger males are more successful than smaller ones at such takeovers[7].

We considered that mounting females might be mistaken for large mating males and so attract only large males that can win in male–male competition. We therefore tested the behaviour of large and small males by presenting them individually with five small and five large dead females glued in the mounting position on the backs of ten live, medium-sized females. As predicted, large males were attracted more to large pairs than to small pairs ($65.5 \pm 10.7\%$ and $34.5 \pm 10.7\%$, respectively; $G=29.7$, d.f. = 8, $n=80$, $P<0.05$), and small males were more likely to approach small pairs than large ones ($71.7 \pm 8.2\%$ and $28.3 \pm 8.2\%$, respectively; $G=16.07$, d.f. = 8, $n=80$, $P<0.05$). By mating preferentially with larger males, females may benefit either through 'good genes' or directly by obtaining resources, as materials are transferred from males to females during mating[7].

Another possible explanation for female–female mounting is that the females are mimicking mating males to reduce male sexual harassment[4,8,9]. However, this hypothesis was rejected because males were more attracted to 'mating' (glued) females ($70.8 \pm 10.9\%$) than to individual females ($29.2 \pm 10.9\%$; $G=23.18$, d.f. = 8, $P<0.05$). Alternatively, females may mount one another in order to appear larger[10] and thus obtain better mates, as males prefer to mate with larger, more fecund females. However, small males are not attracted by mounting females, so this idea can also be rejected.

Our explanation is consistent with all known cases of naturally occurring female–female mounting in insects[1], as males have difficulty distinguishing females and so seek females in copulating pairs. By mounting one another, females can increase their opportunities to mate with large males.

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Environmental toxins

Exposure to bisphenol A advances puberty

Plastics and pesticides are examples of products that contain oestrogenic endocrine-disrupting chemicals, or EEDCs, which can interfere with mammalian development by mimicking the action of the sex hormone oestradiol[1]. For instance, the exposure of developing rodents to high doses of EEDCs advances puberty and alters their reproductive function[2]. Low environmental doses of EEDCs may also affect development in humans[3]. Effects have become apparent in humans over the past half century that are consistent with those seen in animals after exposure to high doses of EEDCs, such as an increase in genital abnormality in boys[4] and earlier sexual maturation in girls[5]. Here we show that exposing female mouse fetuses to an EEDC

at a dose that is within the range typical of the environmental exposure of humans alters the postnatal growth rate and brings on early puberty in these mice.

Oestrogen is a hormone that interacts with other steroids to regulate the normal development of the reproductive system and other tissues. Bisphenol A, a compound that was initially synthesized as a chemical oestrogen[6], is now used as the monomer for the production of polycarbonate plastic products such as baby bottles. Bisphenol A leaches out of such products at a rate that increases with repeated use[7].

Pregnant CF-1 mice ($n=21$ per treatment group) were fed either oil (vehicle) or bisphenol A dissolved in oil at a dose equivalent to that typically found in the environment (2.4 μg per kg), on days 11 to 17 of gestation[8]. Pups were delivered by caesarean section on day 19 to determine their position in the uterus and were reared by untreated foster mothers. The intrauterine position determines fetal hormone levels because endogenous sex steroids are transported from one fetus to another[9]. Mouse fetuses positioned between two males (Fig. 1, 2M) are exposed to the lowest levels of oestradiol, fetuses located next to female fetuses (0M) are exposed to the highest, and females next to one male (1M) are exposed to an intermediate amount[10].

At weaning on postnatal day 22, females treated with bisphenol A were significantly heavier than control females (Fig. 1a), although they had a similar body weight at birth: relative to controls from the same intrauterine position, the weight of 0M females was increased by 22% and that of 1M females was increased by 9%; 2M females were unaffected (Fig. 1d). The findings were virtually identical for male siblings.

On postnatal day 26, females were housed individually but near to males to provide a submaximal level of pheromonal stimulation[11]. After vaginal opening, daily vaginal smears were examined for the presence of completely cornified epithelial cells (a sign of first vaginal oestrus). We found that prenatal treatment with bisphenol A significantly reduced the number of days between vaginal opening and first vaginal oestrus, which is highly correlated with first postpubertal ovulation[12] (Fig. 1c), in 0M females but not in 2M females (Fig. 1f), based on analysis of covariance adjusted for body weight at weaning.

Prenatal exposure to a dose of bisphenol A comparable to levels found in the environment therefore altered postnatal growth rate and reproductive function in female mice, although individual differences in endogenous oestradiol resulting from natural variation influenced the responsiveness of the females to bisphenol A.

There is significant variability in human and animal populations in responsiveness