*Oscheius tipulae**

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Abstract

***Oscheius tipulae* is a common soil nematode of the same family as *C. elegans* (Rhabditidae), which presents the same hermaphroditic mode of reproduction and is easily cultured in the same conditions. *Oscheius tipulae* has been used as a developmental genetic model system to study vulva formation. Compared to *C. elegans*, it has a simpler vulval cell lineage, a reduced competence group and a different mechanism of vulval cell fate patterning. The spectrum of vulval phenotypes obtained in genetic screens differs from that found in *C. elegans*. Its easy isolation from soil and the availability of numerous wild isolates of *O. tipulae* from all over the world facilitate population genetic and microevolutionary studies, especially of the evolution of cell lineage. The *Oscheius* genus also presents many species with interesting evolutionary changes in mode of reproduction, gonad development, body size, etc.**

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1. Phylogenetic relationships

1.1. Phylogenetic position of the *Oscheius* genus

Oscheius tipulae is a nematode of the same family as *C. elegans* (Rhabditidae), commonly found in soil. Its relationship to *C. elegans* is more distant than that of members of the *Caenorhabditis* genus, yet it is more closely related to *C. elegans* than *Pristionchus pacificus* and *Brugia malayi* are (Figure 1). Compared to *C. elegans*, *Oscheius tipulae* shares a common ancestor with the insect parasite *Heterorhabditis* and the vertebrate parasites Strongylida (Blaxter et al., 1998; see The phylogenetic relationships of *Caenorhabditis* and other rhabditids).

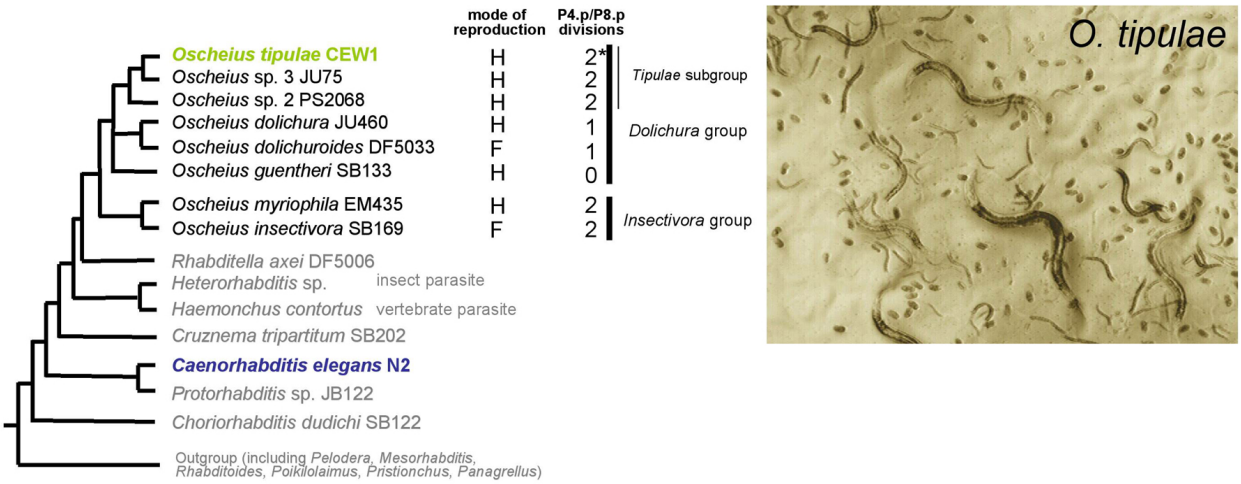

Figure 1. Phylogenetic relationships of some nematodes, showing the position of *Oscheius tipulae*. After Blaxter et al. (1998), Félix et al. (2001), The phylogenetic relationships of *Caenorhabditis* and other rhabditids. Branch lengths have no meaning. Modes of reproduction: H, hermaphrodites and facultative males; F, females and males. Some species in the *Dolichura* subgroup produce F1 hybrids, some of them appearing somewhat fertile (M.-A. F., unpublished). Number of division rounds of P4.p/P8.p : *indicates a polymorphism between 0 and 2 division rounds (Delattre and Félix, 2001a).

1.2. The *Oscheius* genus

The *Oscheius* genus comprises two main subclades (*Dolichura* and *Insectivora* groups; see The phylogenetic relationships of *Caenorhabditis* and other rhabditids; Sudhaus, 1976; Sudhaus and Hooper, 1994; Figure 1). One distinctive character between these two clades appears to be the smaller size of the animals in the *Dolichura* clade (that includes *Oscheius tipulae*) (Flemming et al., 2000) (M.-A. F., unpublished). The *Dolichura* group comprises *Oscheius guentheri*, which is a species with a reduced posterior-gonadal arm (Sudhaus and Hooper, 1994), plus two subgroups, of which two representative species are *Oscheius tipulae* and *O. dolichura*, respectively (see The phylogenetic relationships of *Caenorhabditis* and other rhabditids). Historically, *Oscheius dolichura* (Schneider, 1866) was one of the first nematode species (before *C. elegans*) described to have an androdioceous mode of reproduction (Maupas, 1900; see English translation: http://www.wormbase.org/papers/1900-maupas/index.html).

Oscheius tipulae was first described by Lam and Webster (Lam and Webster, 1971). The species was brought to persisting live culture (strain SB128) and redescribed by Sudhaus (1993). It was called *O. tipulae* because these researchers found it associated with the larvae of the insect *Tipula paludosa* (a dipteran). The CEW1 strain was found by C.E. Winter in soil in São Paulo, Brazil around 1992 (Winter, 1992). Because of taxonomic confusions, the CEW1 strain has been variably named *Dolichorhabditis* sp. or *D. brevesophaga* or *Oscheius* sp. 1 before it was decided to be an isolate of *Oscheius tipulae* (Dichtel-Danjoy and Félix, 2004), which is the valid name.

O. tipulae and its two closely related species, provisionally called *Oscheius* spp. 2 and 3 (Figure 1), are hard to distinguish morphologically, but can be distinguished through mating experiments and molecular features (Félix et al., 2001).

2. Natural populations

2.1. Ecology

Oscheius tipulae is one of the most common nematode species in soil of different (non-desert) parts of the world. It is both widespread and abundant in soil samples. It is mostly (or exclusively) found in the dauer stage (like *C. elegans*), raising the question of whether it proliferates in soil. It was originally isolated from *Tipula* larvae, but this may not be a particularly specific association. *O. tipulae* is more heat-resistant in the lab than *O.* spp. 2 and 3 (Félix et al., 2001) and has been found in tropical as well as temperate regions.

2.2. Isolation and wild genetic resources

Oscheius tipulae is easy to isolate (see Isolation of *C. elegans* and related nematodes). Species of the *Oscheius* genus are characterized in particular by their long rectum (see Figure 5 in Isolation of *C. elegans* and related nematodes).

Available wild isolates can be found at the CGC (http://elegans.swmed.edu/CGC/), the NYU Rhabditidae collection (http://www.nyu.edu/projects/fitch/WSRN/) or in the Félix lab collection (http://www2.ijm.jussieu.fr/worms).

2.3. Population genetics

AFLP (Amplified Fragment Length Polymorphism) analysis on 53 wild isolates from all over the world suggest a genetic diversity at least three-fold higher than that measured with the same method in *C. elegans* (Barrière and Félix, 2005), with no genetic differentiation between continents, yet a clear structure at a smaller scale between three local populations sampled in France (100 km scale), and a relatively high local diversity (D. Baïlle and M.-A. F., unpublished).

3. Basic biology in the laboratory

Oscheius tipulae has the same mode of reproduction as *C. elegans* (self-fertile hermaphrodites and facultative males), and can be cultured and frozen in the same conditions. The lifecycle of the reference strain CEW1 lasts approximately 3 days at 25°C, 4 days at 23°C and 5–6 days at 20°C. *Oscheius tipulae* develops through four juvenile stages and an alternative dauer stage like *C. elegans*. The animals are smaller and slower than *C. elegans*. Eggs are laid at the one-cell stage. Adult hermaphrodites rarely bag upon starvation. The larvae are clear in Nomarski optics. The gonad is syncytial with a central medial region where injected material can diffuse (rachis).

4. Genetics

4.1. Basic forward genetic methods

Oscheius tipulae reproduces through XX hermaphrodites and XO males like *C. elegans*.

The X chromosome non-disjunction rate of the reference strain CEW1 is lower than that of *C. elegans* N2 and males are not easy to induce by heat-shock. However, other *O. tipulae* strains such as PS959 have a higher proportion of males. Males reproduce less well at 25°C and above than at lower temperatures; therefore, crossing plates are better maintained at 23°C (or below).

Mutagenesis and genetic analysis are basically performed as in *C. elegans* (Brenner, 1974). Because the germ line divides later than that of *C. elegans*, mutagenesis is best performed on young adults (rather than L4-stage animals). The concentration of EMS used for *C. elegans* (50 mM) is not as efficient nor as lethal as in *C. elegans*; therefore, mutageneses were performed using an EMS concentration of 100–150 mM, or using TMP-UV irradiation.

4.2. Available mutants

Screens for visible mutants and for mutants with a defective vulva (see below, Section 6.) have been performed. 79 visible markers were isolated: 24 with a Dumpy or Small phenotype, 5 Rollers, 2 Longs, 1 Constipated and 47 Uncs (twitchers, kinkers, sluggish worms, coilers including one dominant allele; Figure 2).

Slightly surprisingly, 35 out of 74 mutants are X-linked (especially 26/43 Uncs compared to 3/23 Dpys, M.-L. Dichtel and M.-A. F., unpublished). Mutants with a Him phenotype (recessive or dominant) were also isolated, as well as one mutant with a partially penetrant left-right inversion of the body (*cov-7*; Delattre and Félix, 2001c; Louvet-Vallée et al., 2003).

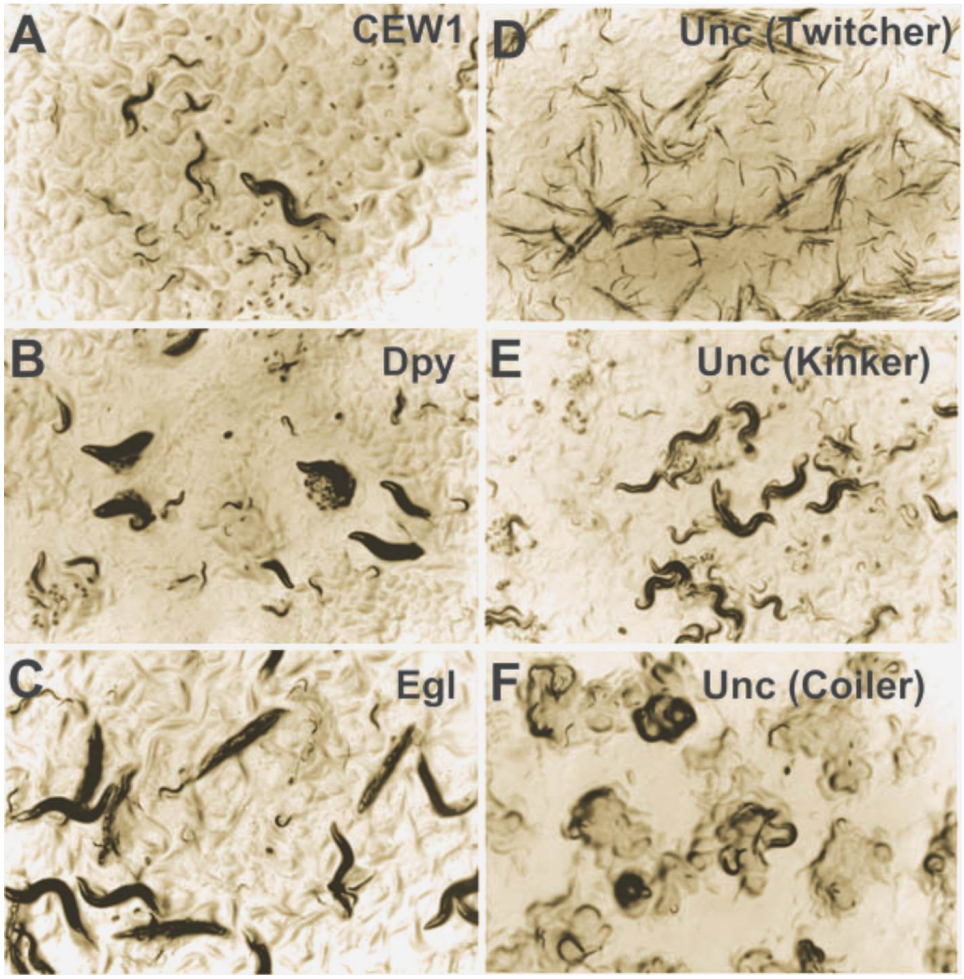

Figure 2. Wild type and some mutants of *Oscheius tipulae*. A. Wild type CEW1. An adult hermaphrodite, embryos and various larval stages are visible. B. Dumpy phenotype (mutant *sy536*). C. Egg-laying defective phenotype (mutant *sy537*) with bags of larvae. D. Twitcher phenotype (mutant *sy486*). E. Kinker phenotype (mutant *sy474*). F. Coiler phenotype (mutant *sy445*).

The apparent haploid number of chromosomes observed by DAPI staining of the germ line is six, as in *C. elegans*. G. Maro, M.-L. Dichtel and M.-A. Félix defined (by genetic complementation or linkage data; unpublished) 6 *dpy* loci, 7 *unc* loci and 2 *lon* loci. The *unc*, *dpy*, and *lon* loci are provisionally designated by a letter to avoid confusion with *C. elegans* loci. Rough linkage data gave six linkage groups, which may or may not cover the six chromosomes:

5 autosomal linkage groups:
A. *unc-a(sy486)* (Twitcher), *unc-c(mf43)*, *unc-d(sy457)*, *unc-e(mf42)*, *dpy-a(mf1)*, *dpy-e(sy517)*, *iov-1(mf86)* (not ordered)
B. *dpy-c(sy471)*, *lon-a(sy475)*
C. *unc-b(mf29)*
D. *dpy-f(sy518)*
E. *con-a(sy462)*

X-linked:
unc-f(sy474), *unc-g(sy485)*, *rol-a(sy472)*, *lon-b(sy502)* (not ordered)

A few mutants are at CGC. Others can be found at http://www2.ijm.jussieu.fr/worms. The gene nomenclature is temporary, since these loci are not homologs to the corresponding *C. elegans* genes. Once their molecular identity is determined, the correct nomenclature is, for example, *Oti-lin-39* for the *Oscheius tipulae lin-39* ortholog.

4.3. Reverse genetics

Injection of morpholinos results in a partial inactivation of the corresponding gene product, for example *Oti-lin-39* (Louvet-Vallée et al., 2003) or *Oti-let-60* (Dichtel-Danjoy and Félix, 2004). However, as in many non-*Caenorhabditis* nematodes, RNA interference does not appear to work efficiently: injection of double-stranded RNA for various genes has not produced any phenotype.

DNA-mediated transformation has been unsuccessful so far, but recent studies in *Strongyloides stercoralis* suggest that using a 3′UTR from the species may be crucial for transgene expression (Li et al., 2006). This remains to be tested in *Oscheius tipulae*. Plasmids injected into the mother's female germ line have been detected by PCR in the F1 progeny (M.-L. Dichtel, unpublished).

5. Genome and molecular biology resources

From rough estimates based on hybridization of a genomic library, the genome size of *Oscheius tipulae* appears similar to that of *C. elegans* (M. Delattre, unpublished). Its GC content was determined to be close to 43% (Ahn and Winter, 2005). Codon biases of highly expressed genes such as vitellogenins appear slightly different from those in *C. elegans* (Winter et al., 1996). Transcription in operons also occurs in *O. tipulae* as in *C. elegans*, with SL1 and SL2 trans-splicing; these SL sequences are similar yet slightly different from those of *C. elegans* (Evans et al., 1997).

Libraries from *Oscheius tipulae* CEW1 include a cDNA library in λZAP II described in Félix et al. (2000) and a genomic library in λFIX II described in Louvet-Vallée et al. (2003) (available on request: felix@ijm.jussieu.fr).

Oscheius tipulae taxon number in sequence databases is 141969; because of changes in the species' name that have been associated with particular strains in the literature (see Section 1.2), some of the sequences may not appear in a direct search for "*Oscheius tipulae*" in databases. In addition, a few dozen ESTs have been sequenced (M.-A. F. and P.W. Sternberg, unpublished).

6. Developmental biology

6.1. Gonad development

Gonad development and morphology of *O. tipulae* are overall similar to those of *C. elegans*, except that germ line divisions are delayed. However, *O. guentheri* presents an interesting case of variable partial reduction of the posterior gonadal arm, which appears to result from a lesser activity of the posterior distal tip cell in its roles in arm migration and germ line proliferation (Félix and Sternberg, 1996).

Anchor cell determination is stochastic and unbiased in *O. tipulae* as in *C. elegans*, but biased towards Z4.aaa becoming the anchor cell in *Oscheius guentheri* (Félix and Sternberg, 1996; Sudhaus and Hooper, 1994).

6.2. Vulva development

Oscheius tipulae has been mostly studied for its vulva development (see Evolution of development in nematodes related to *C. elegans*). Several features make it a particularly interesting comparison to *C. elegans*. Its vulval cell lineage is different, and simpler than that of *C. elegans*. In particular, it is much easier to distinguish the 1° and 2° precursor cell fates by the number of division rounds they undergo (three and two, respectively) (Sommer and Sternberg, 1995; Figure 3). The vulval competence group is composed of P(4-8).p: P3.p is not competent (unlike in *C. elegans*). The adult vulva is formed of six superposed rings, instead of seven in *C. elegans* (Louvet-Vallée et al., 2003). Most strikingly, anchor cell ablations demonstrate that the anchor cell is required after P6.p division for its daughters to acquire a 1° fate: if the anchor cell is ablated in the mid-L3 stage, P(5-7).p all adopt

a 2° lineage pattern. The same spatial pattern of fates is thus induced by different mechanisms in *O. tipulae* and *C. elegans* (Félix and Sternberg, 1997), and recent results suggest that the two-step induction mechanism found in *O. tipulae* is ancestral (Kiontke et al., in preparation).

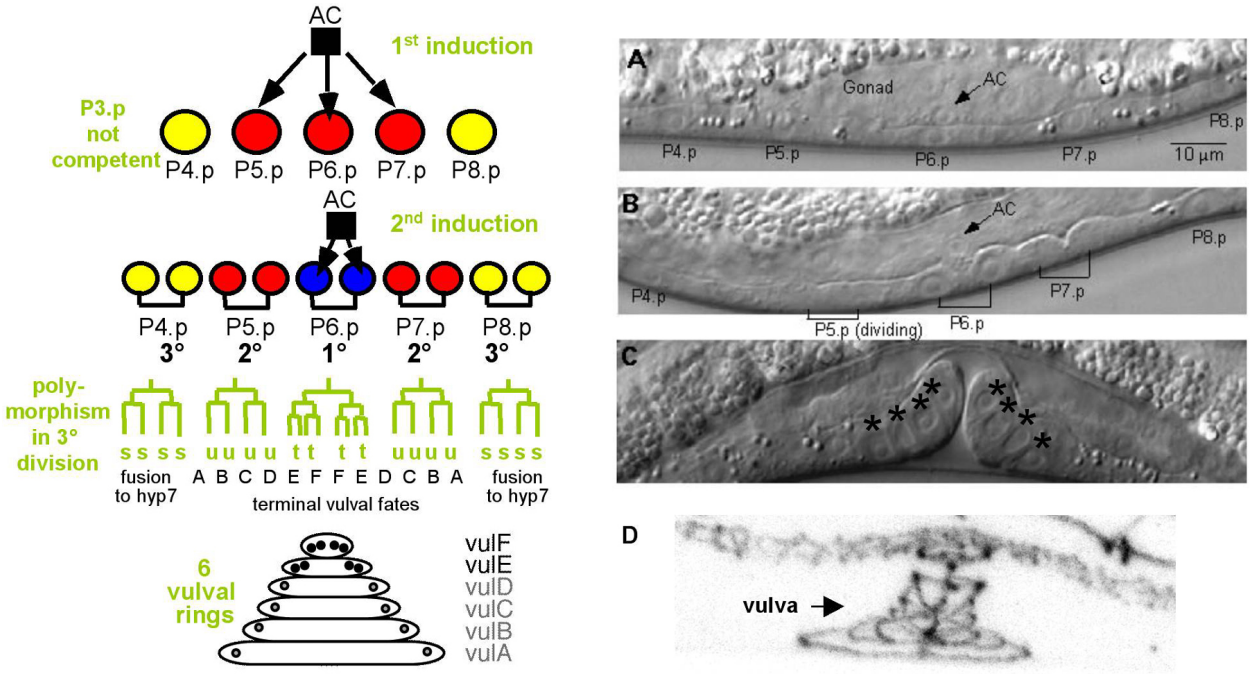

Figure 3. Vulva development in *Oscheius tipulae*. Left panel. Successive stages of vulval development. Differences with *C. elegans* are highlighted in green. AC: anchor cell. S: syncytial fate. U: undivided granddaughter, characteristic of 2° vulval fate. T: transverse (left-right division), characteristic of 1° vulval fate. Right panel. A-C. Nomarski pictures of corresponding stages. In (C), the four granddaughters of P5.p and P7.p are easily visible in the same focal plane on either side of the vulval invagination (stars next to their nuclei). The eight progeny of P6.p are out of focus. D. MH27 staining of cell junctions at the late L4 stage; the uterus cell junctions are visible dorsally to the vulva (top) (courtesy of I. Kolotuev).

O. tipulae has been used for extensive genetic screens for Egl (Egg-laying defective) and Pvl (Protruding vulva) mutants (Dichtel-Danjoy and Félix, 2004; Sommer, 2000). Those with abnormal vulva development were divided in three categories corresponding to successive steps in vulval development:

- Vulva competence group formation and centering : Cov mutants (for Competence/centering Of the Vulva), including a deletion in *Oti-lin-39* (Louvet-Vallée et al., 2003);
- Vulval fate induction: Iov (for Induction Of the Vulva; Dichtel-Danjoy and Félix, 2004);
- Division of vulval precursor cells, without affecting their vulval fates: Dov (for Division Of the Vulva; Dichtel et al., 2001).

Interestingly, the spectrum of phenotypes obtained in vulva mutant screens in *O. tipulae* strongly differs from that obtained in *C. elegans*. For example, the Dov mutations have no equivalent in *C. elegans*, or are at least not found at the same rate. In contrast, Hypoinduced phenotypes have proven difficult to isolate in *O. tipulae*. Also, the 1°/2° fate pattern of Hyperinduced mutants differ between the two species. This comparison of parallel mutagenesis screens on a homologous developmental system shows that the spectrum of phenotypes that can be reached by random mutagenesis evolves (Dichtel-Danjoy and Félix, 2004).

A particularly interesting phenotype of *Oscheius* species is the number of division rounds of P4.p and P8.p: zero in *Oscheius guentheri*, one in *O. dolichura* and *O. dolichuroides* (as in *C. elegans*), but usually two in *O. tipulae*, spp. 2 and 3. However, whereas P4.p and P8.p divide twice in the CEW1 reference strain of *O. tipulae*, this trait is highly polymorphic within an isogenic strain or between wild isolates of *O. tipulae* (Delattre and Félix, 2001a; Delattre and Félix, 2001b). From the analysis of recombinant inbred lines between the two isolates CEW1 and PS959, the difference between them (two versus one division round) is caused by variations at several segregating loci (Delattre and Félix, 2001a). P(4,8).p division is a trait that is easily mimicked by mutation in

CEW1, as shown by the isolation of several mutants with one or zero division round of these cells (Dichtel et al., 2001).

7. Acknowledgement

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