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NEWS AND COMMENTARY

Evolution of sex determination in *C. elegans*

Hidden variation mapped

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Many biological systems are insensitive to a certain degree of environmental variation. Because such systems present some buffering properties (for example, crosstalk between pathways, redundancies and feedback loops), phenotypic variation may occur within the developing system yet be buffered at the level of its output. This buffering allows the accumulation of mutations affecting the system, for example, the activity of signaling pathways, without affecting its end product. Such cryptic genetic variation may have an important function in phenotypic evolution (Wagner, 2005). For instance, it has been proposed that the epidemic aspect of several complex genetic diseases in modern human societies could be explained by the uncovering of cryptic genetic variation in response to the abrupt change of lifestyle in recent generations (Gibson, 2009). Consequently, it is of key interest to study the genetic architecture and molecular nature of cryptic variation that segregates in natural populations. The extent of such cryptic genetic variation has so far been underestimated because of the difficulty in detecting it. In this issue, cryptic genetic variation affecting somatic sex determination mechanisms is revealed among wild isolates of the nematode *Caenorhabditis elegans* (Chandler, 2010). Using a quantitative genetic approach, the author detected several genomic regions underlying this variation. This work opens interesting perspectives for understanding the evolution of sex determination at the intraspecific level and highlights the importance of considering cryptic variation when studying an apparently invariant system.

Somatic sex determination in *C. elegans* is a good example of a robust system: XX animals (hermaphrodites) develop female somatic organs, whereas XO animals (males) develop male somatic organs, independently of wild genetic background or environmental conditions (Figure 1). To uncover the presence of genetic variation in this system, Chandler (2010) used a strategy that has already been used in previous studies of cryptic genetic variation (Gibson *et al*., 1999; Milloz *et al*., 2008; Dworkin *et al*., 2009): the system was sensitized by introducing mutations, here two mutations of the sex determination pathway (*tra-2(ar221ts); xol-1 (y9)*), into the genetic background of five wild *C. elegans* isolates (N2, CB4856, MY2, AB1 and JU258). In these five introgressed strains, two quantitative traits were measured at different temperatures (the *tra-2* mutation is temperature sensitive): (i) the proportion of XX individuals presenting male somatic gonad morphology and (ii) the degree of tail masculinization of XX individuals using a mean tail score on a 1 to 6 scale (Figure 1). The author observed different trait values depending on wild genetic background. For instance, at temperatures at which penetrance is incomplete, masculinization is slightly greater in the background of N2 compared with that of CB4856. Chandler (2010) thus uncovers cryptic variation among *C. elegans* wild isolates that does not affect somatic sexual fates in a wild-type context, but alters sexual structures when the system is sensitized.

Chandler (2010) then analyzed the genetic architecture of the cryptic variation uncovered between the N2 and CB4856 genetic backgrounds. He performed a quantitative trait locus (QTL) analysis using a set of 66 recombinant inbred lines (RILs) from the two corresponding strains that carry the double mutation *tra-2(ar221ts); xol-1(y9)* (Figure 1). The objective of QTL mapping is to detect genomic regions associated with a significant part of the phenotypic variation, either in a synthetic population (for example, F2, RIL) or in a natural population. Polymorphic genetic markers are genotyped and the trait of interest is measured in individuals of the study population. With these genotyping and phenotyping data, different statistical methods are then used to estimate the number and position of the genetic loci involved in the phenotypic variation (QTLs), as well as the intensity and sign of their effect, and possible epistasis between them. Chandler (2010) chose to use RILs, an approach which is becoming popular for QTL analyses in *C. elegans* (Kammenga *et al*., 2008; Rockman and Kruglyak, 2009). The construction of a large set of RILs is easy in *C. elegans* because of its short generation time and selfing mode of reproduction. The main advantage of RILs is the low genetic variation within lines, due to inbreeding. Therefore, compared with F2 populations that are partially heterozygous, the phenotype of RILs can be measured from many genetically identical individuals, which increases the sensitivity of QTL detection. RILs can also be kept frozen in *C. elegans*, so a given set can be used for several studies focusing on different traits. By using a multiple interval mapping method, Chandler (2010) detected several loci involved in gonadal sex ratio and tail score variations, which confirms the genetic basis of the cryptic variation. One QTL located on chromosome IV displays a particularly large effect, with the N2 allele leading to stronger masculinization than the CB4856 allele.

The next important step will be to identify the nucleotide polymorphisms responsible for this cryptic variation. These polymorphisms may not necessarily affect known components of the sex determination pathway, as cryptic variation could subtly affect sexual fate through genes whose effects were undetectable in classical genetic screens. Indeed, Chandler (2010) found that some QTLs (such as the chromosome IV QTL) map in intervals containing known sex determination genes, whereas others do not.

One key question will then be to determine whether this genetic variation could affect phenotypes other than the sexual fate of somatic tissues. Cryptic variation may in principle accumulate neutrally, as it does not affect the phenotypes of interest—here the sexual fate of somatic tissues. However, cryptic variation may also evolve under selection for another phenotype. Finding the molecular nature and the phenotypic effect of QTLs involved in cryptic variation may therefore help to understand the evolutionary forces behind the accumulation of cryptic variation.

A particularly interesting phenotype that is possibly coregulated with sex determination in the soma is sex determination in the germ line. In the *Caenorhabditis* genus, evolution of sexual fates occurred in the germ line and not in the soma, even though both systems rely on a shared regulatory pathway (Zarkower, 2006; Ellis and Schedl, 2007). First, at the interspecific level, evolution

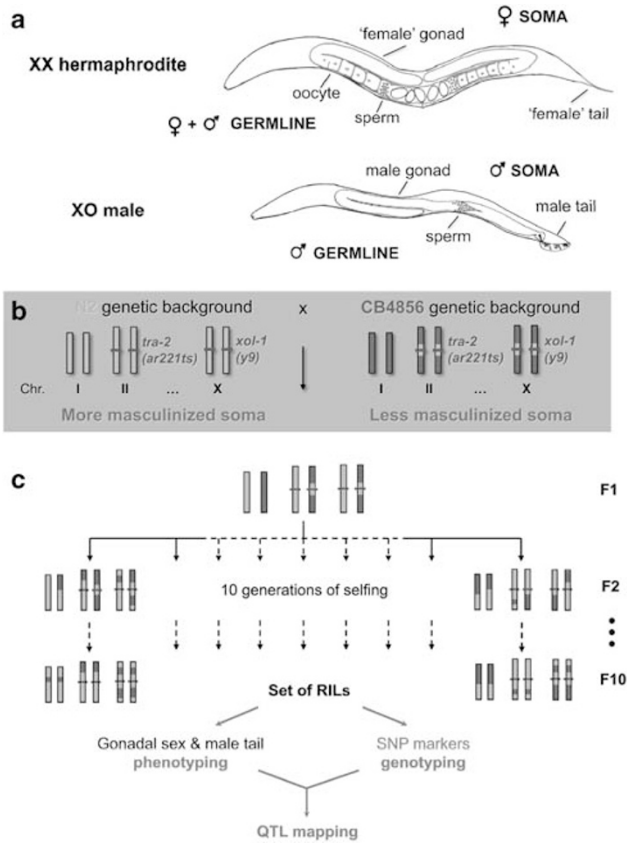

Figure 1 Uncovering cryptic genetic variation affecting somatic sex determination in *C. elegans*. (**a**) Sexual differences between wild-type hermaphrodite and male. Somatic structures differing between sexes are shown in red and differentiated cells in the germ line are shown in green. Hermaphrodites present a female-like soma, but produce both male (early in life) and female (throughout adulthood) gametes. Males harbor male organs and generate only sperm. Several other tissues (not represented) differ between sexes, notably, the nervous system and musculature. (**b**) Introgression of two mutations in the wild isolates N2, and CB4856 uncovers the presence of cryptic genetic variation. The two mutations *tra-2(ar221ts)* and *xol-1(y9)* originally obtained in the reference N2 background were introgressed by repeated outcrosses in the CB4856 background. The resulting strains exhibit different degrees of somatic masculinization, revealing cryptic genetic variation within *C. elegans* (Chandler, 2010). (**c**) Recombinant inbred lines (RILs) construction scheme. A set of 66 RILs was used to map the cryptic genetic variation uncovered between N2 and CB4856 (Chandler, 2010). Possible chromosome genotypes are represented with N2 background colored in orange and CB4856 background colored in blue. A full color version of this figure is available at the *Heredity* journal online.

of germline sexual fates forms the basis for the evolution in reproductive mode. Most *Caenorhabditis* species are gonochoristic (male/female), whereas *C. elegans* and *C. briggsae* are androdioecious (Haag, 2005), with a vast majority of XX hermaphrodite individuals, and some rare XO males. XX females from gonochoristic species and XX hermaphrodites from androdioecious species both present a female soma, but differ in the germ line: while a female generates only oocytes, a hermaphrodite's germ line first produces a limited stock of sperm at the beginning of adulthood, before irreversibly switching to oocyte production. Second, at the intraspecific level, the regulation of germline sexual fate also varies in *C. elegans* hermaphrodites. The regulation of the sperm-to-oocyte transition determines the number of sperm and thus brood size. Even though sperm number has rarely been directly quantified, variations in self-brood size have been detected for different *C. elegans* wild isolates and in different laboratory environments (Hodgkin and Doniach, 1997; Harvey and Viney, 2007). Thus, in contrast with somatic sexual fates, the switch in germline sexual fates is plastic and may vary with wild genetic background. Interestingly, increased brood size through increased sperm number may not always be selectively advantageous, as it is associated with a delay in oogenesis onset and thus a longer generation time (Hodgkin and Barnes, 1991). Sperm number is likely to be a trait under strong selective pressure, with an optimum dependent on the environmental context. In sum, a polymorphism that shows a cryptic effect on sexual fates in somatic tissues could affect the germ line in a non-cryptic way, for example, by tuning the number of sperm produced by hermaphrodites. Therefore, determining the effect of the somatically cryptic variation on germline sexual fate may indicate how the evolutionary forces acting on the germ line could drive the fast evolution of genes involved in sex determination (Haag, 2005) and perhaps result in cryptic variation in somatic sex determination.

Conflict of interest

The authors declare no conflict of interest.

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