

East Asian and Oceanic populations. Other interpretations of the fossil record have found no evidence of admixture between Neandertals and modern humans [10]. This is not surprising, as it is unclear whether 1–4% admixture would be detectable in skeletal morphology; however, shared skeletal features between Neandertals and Eurasians to the exclusion of Africans should be sought.

The question is then: when and how did admixture occur? Green *et al.* [1] propose two alternative scenarios. First, they note that ancient substructure within Africa could create such a pattern in the absence of admixture. If African genetic diversity was structured at the time the ancestors of Neandertals left Africa to colonize western Eurasia and this structure persists until today, then some African populations might be more closely related to Neandertals than others. If this population was also the source of the later modern human migration out of Africa, then Eurasians may appear to have more affinities to Neandertals than to some Africans. Further sampling of Africa would then be expected to reveal Africans with similar relatedness to Neandertals as the Eurasian samples. However, Green *et al.* [1] favor a second scenario which involves admixture between Neandertals and modern humans early during the exodus from Africa. In this scenario, no Africans will be found with genetic signs of Neandertal admixture (Figure 1B).

We propose a third alternative. The paleontological and archaeological records suggest that modern humans and Neandertals overlapped in the Eastern Mediterranean region around 100 thousand years ago during a time when the African faunal zone extended temporarily into the Middle East. The range of modern humans then likely contracted back into Africa, severing contact with Neandertals, before finally expanding their range out of Africa around 50 thousand years ago [11]. Admixture may not have been possible during this time because a southern route out of Africa through the Arabian peninsula [12] would not have put the populations in contact. Any admixture would have occurred prior to the expansion of modern humans out of Africa between East Africans and Neandertals (Figure 1C). If this is correct, Neandertal genes will be found at low frequency in East Africans and

perhaps others. These low-frequency Neandertal genes may then have been pushed to high frequency or fixation in the out of Africa populations through the iterated founder effect associated with range expansions [13].

To distinguish between ancient substructure or admixture within or outside Africa we need to better understand African genetic diversity. Green *et al.* [1] laudably built a comparative data set consisting of five complete human genomes. However, two individuals cannot possibly represent African diversity. Recently two additional South African genomes were fully and three more partially sequenced, revealing 1.3 million novel variants [14]. It is also known that African genetic diversity is significantly structured [15]. This suggests the majority of African genetic diversity is yet unknown. These are population genetic questions that will require population samples to resolve. Sequencing costs have dropped to the point where population genomics is becoming feasible. As the most genetically diverse and least understood, African populations should be given priority. Now that the Neandertal genome has been well characterized, it is clear that if we are to fully understand the relationship between Neandertals and living people, we need to better understand the genomic diversity of living humans.

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Evolutionary Biology: The Origins of Two Sexes

Evidence is accumulating that in the green algae the evolution of female and male gametes differing in size — anisogamy — involves genes linked to the mating-type locus, as was predicted theoretically.

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Anisogamy has evolved independently in several different groups of

organisms, but the green algae seem particularly promising for studying this evolutionary change [1] because some taxa, including *Chlamydomonas* species, are isogamous, with no

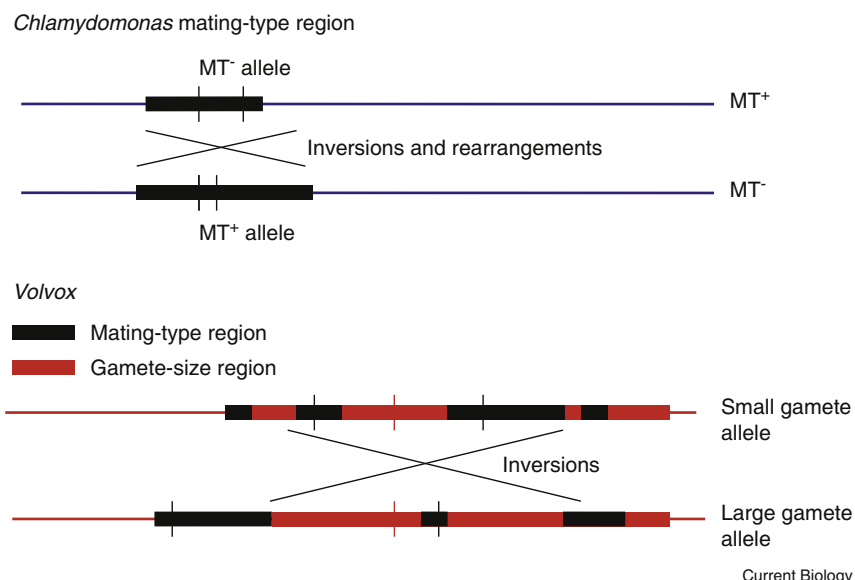


Figure 1. Diagram of the *C. reinhardtii* and *V. carteri* mating type (MT) regions.

The diagrams are not to scale, but indicate the non-recombining regions as thick bars (with different sizes in the + and – alleles of the MT region, resulting from rearrangements of the genome in these regions). Two loci determining the mating type are indicated as thin black vertical lines (again, not intended to show their true locations in these regions). In *V. carteri*, the non-recombining regions of the + and – mating types are both very large because of the inclusion of genes that are not part of the *C. reinhardtii* MT regions. These added genes are inferred to include genes that control gamete size, and rearrangements have caused them to be interspersed among MT region genes.

gamete size differences, while others, including *Volvox* species, are anisogamous. Anisogamy probably evolved as a result of large zygotes having a survival advantage, opening the way for individuals producing smaller gametes to gain an advantage through high gamete output [2,3]. If large zygotes are advantageous, small gametes that fuse with other small gametes will clearly be highly disadvantageous. An additional part of the theory for the evolution of anisogamy is therefore the prediction that there is likely to have been a pre-existing mating-type system preventing matings between identical types, and that a gamete size-change mutation will be unable to spread in the population unless it is genetically linked to the mating-type locus [4]. A mutant producing large gametes, say, will then produce gametes that are guaranteed to fuse only with gametes of the other mating type, and these will be the original (smaller) size. Once such a mutation has spread in the population, it will clearly be advantageous for recombination to be suppressed in the region, so that the linkage between the appropriate mating-type

allele and the sex type (large gametes or small) is maintained, and the disadvantageous recombinants are rarely produced (Figure 1). Evidence has now been reported for linkage between the mating-type locus and a gene or genes controlling a gamete size difference in a green alga [5].

These predictions are testable in the green algae because there is already a wealth of information about the mating-type locus (MT) in *Chlamydomonas* species. In *C. reinhardtii*, this locus is within a multi-gene region with suppressed recombination. The suppressed recombination in this region probably evolved for reasons similar to these just explained for gamete size differences. In a mating-type system that works by ‘lock-and-key’ recognition, with two component proteins that must interact, each component must be associated in the genome with the appropriate version of the other component that ensures that it will not mate with itself, and this is the case in all mating-type systems and incompatibility systems whose molecular genetics have been

understood so far, in fungi and flowering plants (for example [6–9]), and in the primitive chordate animal *Ciona intestinalis* [10].

In *C. reinhardtii*, at least two genes in the MT region are known to be involved: the *MID* gene, involved in specifying the *MT*[–] mating type, plus at least one other gene [11], although the details of the processes involved remain unknown. There is a large non-recombining MT region, more than 200 kilobases long, although the size differs between the two MT alleles, *MT*⁺ and *MT*[–]. These alleles differ by multiple genome rearrangements which may have been involved in suppressing recombination between the component genes. This region also includes many genes that may have no mating-type roles, but are simply trapped within the region, so that the *MT*⁺ and *MT*[–] haplotypes have alleles that are diverged, similar to the differences between Y and X chromosomes in diploid species with sex differences [12]. But although the MT region is sometimes characterised as ‘sex-determining’ [13], this species not only does not have male and female sexes, it also does not even have a gamete size difference.

In contrast, *Volvox carteri* and some other, but not all, *Volvox* species [1] have large, non-motile female gametes and small male gametes (this is called oogamy). It has now been discovered that, as predicted, the genes determining this difference in *V. carteri* are linked to the MT locus [5]. *Volvox* species are very distantly related to *Chlamydomonas*, and probably diverged ~200 million years ago [1,14]. Nevertheless, sequencing of *V. carteri* genomic DNA has identified a region containing several genes also known to be in the *C. reinhardtii* MT region, and genetic mapping in *V. carteri* showed that this region includes the gamete size-determiner.

The region of the *V. carteri* genome includes much more DNA than the *C. reinhardtii* MT region; interestingly, this is partly because it includes many genes that are not part of the *C. reinhardtii* MT region, and partly because it contains more repetitive sequences,

and sometimes longer introns, reminiscent of the way Y chromosomes differ from X chromosomes (for example [15,16]). Although the genes determining the gamete size difference have not been shown to be present or identified, several genes in the *V. carteri* MT region are expressed mainly during gamete production, and some of these are present only in the male haplotype, and others only in the female haplotype — that is, they are ‘gender-linked’ (called ‘gender-limited’ in [5]). These findings suggest that a new gamete-size-controlling region with recombination suppression has been added adjacent to an ancestral MT region, as predicted.

There are, however, some problems with this simple interpretation. A first worrying finding is that the two known genes that are expressed only in *MT*[−] haploids of *C. reinhardtii* — *MID* (see above) and *MTD1* [11], which may boost *MID* expression [13] — are expressed in vegetative cells as well as gametes [5]. In *Pleodorina*, another Volvocine, the *MID* gene is found only in males, suggesting that males evolved from an *MT*[−] strain, and that *MID* is linked to the gene controlling gender [1,17,18].

A second puzzle is that many genes in the *V. carteri* MT region show much greater divergence between the two haplotypes than that seen in *C. reinhardtii*. This is not what one would predict if the *Volvox* system evolved from a smaller, older MT region still present in *C. reinhardtii*. In that case, the average inter-haplotype divergence should then be smaller in the *Volvox* system (Figure 2) — divergence for the MT region genes should be similar (reflecting their common ancestry), but genes that were incorporated into the non-recombining region after the mating types were established will have had less divergence time, like parts of the mammalian Y chromosome that stopped recombining with their X-linked counterparts more recently than others [12]. The *V. carteri* MT region indeed includes a part with low divergence, perhaps added recently to the non-recombining region, and (as expected) there is no difference between sequences sampled from the two genotypes at

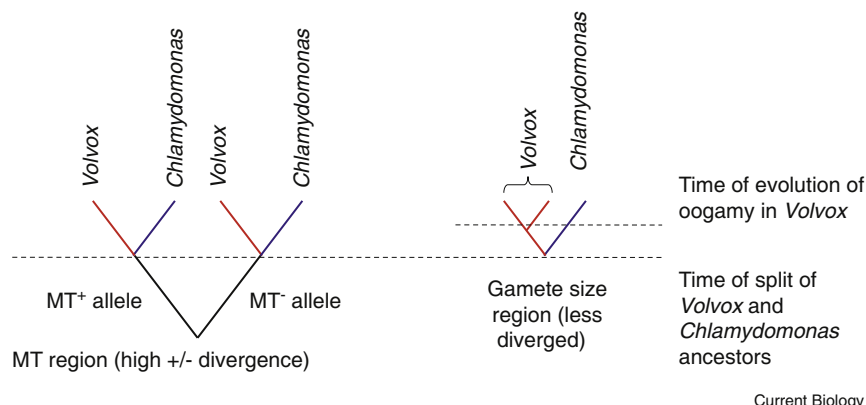


Figure 2. Expected divergence times.

Expected divergence times between the MT region genes of *MT*⁺ and *MT*[−] haplotypes of *Chlamydomonas* and *Volvox*, and for genes not in the *C. reinhardtii* non-recombining MT region (which should have lower inter-haplotype divergence in *Volvox*, if they became part of the non-recombining region more recently than the MT genes).

a gene in the recombining flanking region. However, for genes that are present in both the sexual haplotypes of *Volvox*, divergence between the two copies is generally extremely high (synonymous *MT*[−]/*MT*⁺ divergence in the 11 genes that were sequenced from both mating-type haplotypes in both the species was about 3.4% for *C. reinhardtii*, 22 times less than in *Volvox*, and non-synonymous *MT*[−]/*MT*⁺ divergence often exceeds 20% in *Volvox*, versus at most 4% in *C. reinhardtii*). Perhaps, therefore, the *C. reinhardtii* MT region evolved relatively recently, and independently of that in *Volvox*. Isogamous species more closely related to *Volvox* will need to be studied to determine whether this interpretation is correct.

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