amnesiac, the same genes required in adult fly learning. Recently Khurana *et al.* [4] found that *dunce* is involved in short-term memory in the same odor-heat shock paradigm Robinson *et al.* [1] used to study alcohol dependency.

The *dunce*, *rutabaga*, *radish*, and *amnesiac* genes encode components of the cAMP cascade that are also well-known ethanol targets [3,4]. *Dunce* encodes a cAMP-specific phosphodiesterase; *rutabaga* encodes an adenylyl cyclase; and *amnesiac* encodes an adenylyl cyclase-activating neuropeptide that has been shown to be hypersensitive to ethanol. The identity of *radish* was unknown until Guan *et al.* [5] recently discovered that the radish protein is a PKA phosphorylation target that travels between the nucleus and cytoplasm. These authors also showed that *dunce*, *rutabaga*, *radish*, and *amnesiac* mutation each disrupts specific aspects of synaptic connectivity. This finding increased our understanding for the role of the members in the cAMP cascade in synaptic reorganization. Connecting the cAMP cascade back to alcohol, a recent study demonstrated a key role for *rutabaga* in ethanol self-administration: Xu *et al.* [6] showed that flies preferred food with ethanol to food without ethanol and this preference was dependent on expression of *rutabaga* in the mushroom bodies. This shows an interesting convergence of ethanol self-administration and olfactory associative memory behavior onto *rutabaga* in the mushroom bodies. Future studies using Robinson *et al.*'s [1] model might reveal more about the relationship of the two.

Humans often drink too much because they find being drunk rewarding in some way. Do *Drosophila* find being drunk rewarding? Will they turn to drink to drown their sorrows? Two recent studies [7,8] have shown remarkable parallels between ethanol consumption in flies and humans. Kaun *et al.* [7] developed a conditioned place preference paradigm for flies and showed that flies perceive intoxicating levels of ethanol as rewarding. Flies were exposed to two odors, one in the presence of intoxicating levels of ethanol vapors, the other without. After training, flies preferred the odor that had been paired with the high level of ethanol! As in mammals this preference was dependent on dopamine. In this paradigm flies were exposed to ethanol vapor by the experimenters, but the question remained as to what might make flies voluntarily consume ethanol.

A clever study by Shohat-Ophir *et al.* [8] indicated that, like humans, flies try to drink their troubles away! One group of male flies was exposed to one-hour sessions of rejection by already mated females three times a day for four days, and another group to six-hour sessions of mating with multiple receptive virgin females for four days. Flies were then exposed to a two-choice task where they could consume food with or without ethanol. As you might guess, flies that had experienced repeated rejection consumed significantly more ethanol than successful flies! Shohat-Ophir *et al.* [8] showed that this increase in ethanol consumption was directly linked to an increase in expression of a neuropeptide, NPF, as failure at mating led to a decrease in NPF expression, while decreases in NPF expression led to increased ethanol consumption. The mammalian homolog of NPF is neuropeptide Y (NPY). In the nematode *Caenorhabditis elegans*, the NPY receptor homolog NPR-1 regulates ethanol behaviors [9]; in mammals stressful experiences regulate NPY levels, and NPY-deficient rats drink more ethanol than controls [10]. Thus, across phylogeny, the relationship between social stress, NPY and ethanol consumption seems to be remarkably conserved.

Will the rejected male flies become addicted to ethanol? Will they show cognitive dependence such that their ability to learn and remember will become dependent on the presence of ethanol? Will they lose their jobs and beat their larvae? Stay tuned — drunk flies can teach us a lot about the mechanisms underlying the debilitating aspects that ethanol has on human behavior!

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Speciation: Clash of the Genomes

Complete genomes of hybridizing bird species demonstrate the importance of the sex chromosomes, telomeres and centromeres to the initial stages of speciation.

Bettina Harr[1,*] and Trevor Price[2]

Living in central Sweden in the 18th century, Carl Linnaeus apparently did not see the Pied flycatcher (*Ficedula hypoleuca*), now one of Sweden's most common breeding birds (Figure 1) [1]. Nor would he have seen the Collared

flycatcher (*Ficedula albicollis*) when he visited the Baltic island of Gotland, where it currently breeds and hybridizes with the Pied flycatcher (Figure 1). The two species seem to have colonized these locations only within the last 200 years [1]. Perhaps because he did not see the birds, Linnaeus did not describe the Collared flycatcher at all and muddled his description of the Pied flycatcher. But his confusion on the Pied flycatcher also stemmed from previous suggestions that it was the same species as the Blackcap, *Sylvia atricapilla,* and changed into Blackcap plumage in the winter [1]! Work recently reported in *Nature* [2] shows just how far we have come since Linnaeus's time. Ellegren *et al.* [2] analyze about 90% of the genomic DNA sequence of these two flycatchers, making them the 4th and 5th in the list of published bird genomes (following the chicken, turkey, and zebra finch; a Darwin's finch lurks at: http://gigadb.org/darwins-finch/).

The range shifts of the flycatchers epitomize a general process that must have been going on at least since the beginning of the Pleistocene about 2.5 million years ago. Associated with the ebb and flow of the ice sheets, populations became sequestered to climatic refuges and presumably diverged genetically, then expanded ranges and met in contact zones leading to gene exchange. In what has become something of a paradigm shift in our understanding of the way speciation occurs, it appears that, once some genetic divergence has taken place, not all parts of the genome are equally likely to cross from one taxon to the other when they come back into contact [3]. Some parts of the genome rapidly become genetically incompatible, other parts have no detectable effect on fitness, and yet others may be actually favored in the other taxon. The result is that if related species have had repeated opportunities for hybridization in the past, their genomes may consist of a mosaic of divergence times [3,4].

The Collared flycatcher and Pied flycatcher are closely related and hybridize where they meet (Figure 1) at a frequency of up to 10% [5,6]. However, the hybrids have low fitness: Wiley *et al.* [6] studied populations on the Baltic island of Öland and found that the number of grand-offspring from a hybrid pairing was less than 3% that from conspecific pairs. In their study Ellegren *et al.* [2] obtained a historical estimate of gene flow between these two species by applying a coalescence model to DNA sequence data from 24 neutral nuclear loci (10 individuals per species). Gene flow does appear to have occurred during the evolutionary history of these two species, but the rate is estimated to be low and asymmetric, with the equivalent of less than one migrant individual every three generations from the Pied flycatcher population into the Collared flycatcher population, and much less than that from the Collared flycatcher population into the Pied flycatcher population. However, despite the presence of gene flow over a substantial amount of evolutionary time, several segments in the genome are very strongly differentiated between the species, indicating essentially no gene flow at all in these particular regions, which is in contrast to the remainder of the genome. These "islands of differentiation" [4] are of considerable interest, as they may contribute to reproductive isolation, i.e., hybrids carrying genetic material in these regions from both species have particularly low fitness. The strongly differentiated regions are the centromeres and telomeres of many of the autosomes, as well as the Z chromosome (in birds the sex chromosomes are labeled Z and W, analogous to X and Y of mammals, except the female is the heterogametic sex (ZW) and the male is the homogametic sex (ZZ)). Although Ellegren *et al.* [2] note several methodological issues that may inflate the importance of the centromeres and telomeres, the observed pattern is strong. What is special about these regions?

During meiosis, the centromeres and spindles together determine which chromatid goes into the polar bodies rather than the egg of the female. Any new mutation that promotes a particular centromere's entry into the egg is powerfully favored and should, therefore, rapidly increase in frequency [7]. A similar argument can be made for telomeres, which play a unique role in anchoring chromosomes to the inner nuclear membrane during early meiosis, thereby affecting segregation [8]. Several models have been developed which predict that evolution at these sites should lead to a failure of meiosis when different copies are brought together into the same genome, thereby creating reproductive isolation. Indeed, in a review, Henikoff *et al.* [9] state that "*speciation is an inevitable consequence of centromere evolution*". However, it is something of a leap to go from differentiation to reproductive isolation and a direct causal link will be difficult to establish for the Collared flycatcher and the Pied flcyatcher. This is because the regions that affect reproductive incompatibility need to be mapped genetically,

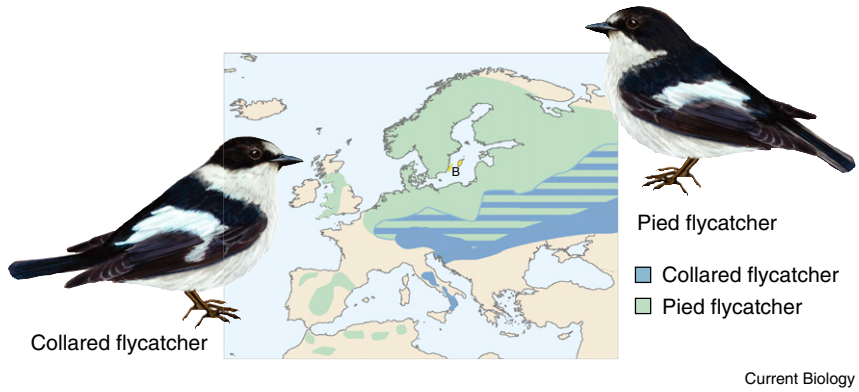

Figure 1. Distribution of the Collared flycatcher and Pied flycatcher.
Both species breed and hybridize on the Baltic islands of Gotland and Öland (where they were studied by Ellegren *et al*. 2012 [2]), as indicated in yellow and by the letter B. Drawing by Emiko Paul, reproduced with permission of Roberts & Co from [17].

but mapping in natural populations requires a substantial number of late-generation hybrids [10]. Ellegren *et al.*'s [2] study sets the stage for analyses on other hybridizing species, and in particular, hybridizing species that are at an earlier stage in the development of reproductive isolation than these two flycatcher species.

The Z chromosomes are strikingly differentiated between the Collard flycatcher and the Pied flycatcher. Sex chromosomes in general are widely appreciated to play an important role in speciation [11]. Sex chromosomes may evolve more rapidly than the autosomes because of a higher mutation rate and/or selection pressures. Any given Z chromosome spends two-thirds of its time in a male, whereas an autosome spends only half of its time in a male. The male germ line experiences a higher mutation rate than the female because of the multiple cell divisions in sperm production. With respect to selection, because the Z is in a male for two-thirds of its history, selection for male function can override selection for female function. However, the exposure of recessives in the female means that mutations which favor female function can increase even when detrimental to males. Factors such as these may lead to various conflicts, driving faster evolution of the Z chromosomes than the autosomes. In the case of the flycatchers there is some evidence for faster rates of sex-chromosome evolution: Z-chromosome incompatibilities may affect male fertility more than autosomal incompatibilities [12]. However, probably more important than faster evolution is that, even if reproductive incompatibilities were to accumulate at a similar rate on the Z and the autosomes, any incompatibility that is recessive and located on the Z is exposed to selection in the female hybrid. For example, a recessive mutation on the Z from one species may interact detrimentally with a dominant mutation on an autosome from the other species, a mutation on the W, and any special maternal factors (cytoplasm, mitochondrial DNA; a feature of F1 hybrids that is unique to species in which the female is the heterogametic sex is that the cytoplasm comes from one species and the exposed sex chromosome comes from the other).

In the theory of divergence in the face of gene flow, an additional factor promoting differentiation is that of chromosomal inversion differences. Because inversions inhibit recombination, any incompatibility in the inversion should restrict gene exchange between the taxa at all other associated loci, with consequent build-up of strongly differentiated blocks [13,14]. Ellegren *et al.* [2] found no evidence that inversions separate the Collared flycatcher from the Pied flycatcher. Indeed, an intriguing finding from their work is the presence of few chromosomal inversions in these species, even when compared to the chicken, from which they probably diverged 80–100 Ma [15]. This, as well as similar findings in the Great Reed warbler, *Acrocephalus arundinaceus* [16], suggests that inversions play a limited role in bird speciation. That conclusion is premature. Several avian groups do show many inversion differences [17]. For example, the family of finches to which the zebra finch belongs carries many inversions, both within and between species. The zebra finch itself is polymorphic for a large inversion on the Z chromosome. It is possible that the individual whose genome was the first of any songbird to be sequenced [18] may have been heterozygous for this inversion [19].

We have come a long way since Linnaeus confounded the Pied flycatcher and the Blackcap, but may be in a similar state of uncertainty in the genomics era. As exemplified by Ellegren *et al.*'s [2] study, things are changing quickly and perhaps within the next ten years we will have complete genomes of the majority of the world's birds. By 2022, we may have learnt as much, or more, about speciation than the sum total of what we now know.

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