of fresh water. This is enough to balance our measured salinity with a mean sea-level change of 135 m. Increases in ice-shelf volumes can also balance the salt budget. Given a total volume of 0.7×10^6 km^3 for all the Antarctic ice shelves (*50*), there would have to be seven times this amount of floating ice at the LGM to balance our data with the sea-level constraints.

47. G. H. Denton, T. J. Hughes, in *The Last Great Ice Sheets*, G. H. Denton, T. J. Hughes, Eds. (Wiley, New York, 1981), pp. 437.
48. B. P. Boudreau, *Diagenetic Models and Their Implementation* (Springer-Verlag, Berlin, 1997).
49. P. A. Domenico, F.W. Schwartz, *Physical and Chemical Hydrology* (Wiley, New York, 1990).
50. D. J. Drewry, Ed., *Antarctica: Glaciological and Geophysical Folio*, sheets 2–9, Scott Polar Research Institute, University of Cambridge, Cambridge, UK (1983).
51. S.-T. Kim, J. R. O'Neil, *Geochem. Cosmochim. Acta* **61**, 3461 (1997).

52. We thank K. Cuffey for insightful discussions about hydrology and E. Boyle and an anonymous reviewer for helpful comments. E. Boyle is thanked for inspiration and continued encouragement. E. Goddard provided assistance with lab work. Supported by NSF grant numbers OCE-0096814 to J.F.A. and OCE-0096909 to D.P.S.

17 July 2002; accepted 29 October 2002

Hybrid Speciation in Experimental Populations of Yeast

Duncan Greig,[1,2] Edward J. Louis,[3] Rhona H. Borts,[3] Michael Travisano[2]*

Most models of speciation require gradual change and geographic or ecological isolation for new species to arise. Homoploid hybrid speciation occurred readily between *Saccharomyces cerevisiae* and *Saccharomyces paradoxus*. Hybrids had high self-fertility (about 82%), low fertility when backcrossed to either parental species (about 7.5%), and vigorous growth under different thermal environments that favored one or the other of the parental species. Extensive karyotypic changes (tetrasomy) were observed in the hybrids, although genic incompatibilities accounted for 50% of the variation in self-fertility.

Speciation is thought to arise by gradual evolution of genic incompatibilities (*1*), ecological specialization (*2, 3*), or chromosomal differences (*4*) that prevent mating or cause inviable or infertile hybrid offspring (*5*). Rapid species formation can potentially occur by hybridization; however, the degree of reproductive isolation between potential new hybrid species and the two parental species is a major limiting factor. Hybrids must be self-fertile and sufficiently reproductively isolated to maintain a distinct lineage, but reproductive barriers between parental species must not preclude the initial hybridization. In postzygotically isolated species, where hybrids are typically inviable or sterile (*6*), these conflicting requirements can be achieved by a doubling of chromosome complement in the new species to produce an allotetraploid (*7*). Potentially, these requirements can also be met by maintaining chromosome number (homoploid hybrid speciation) (*8, 9*), but this mechanism is very uncommon in plants and unknown in animals (*10*).

Saccharomyces yeast species are postzygotically isolated, because hybrids form readily but are sterile, producing only ~1% viable gametes (spores) (*11–13*). However, populations of yeast can be very large (>10^8), and viable gametes can be easily obtained. Moreover, the ability of *Saccharomyces* gametes to divide and switch mating type allows for autofertilization (gametophytic selfing) and, potentially, for instantaneous homoploid hybrid speciation. We investigated this potential with *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* and measured the effects of intrinsic incompatibilities (hybrid sterility and infertility) and extrinsic incompatibilities (relative fitness of hybrids under different environmental conditions) (*14*).

First, we crossed *S. cerevisiae* and *S. paradoxus* and isolated 80 independent viable haploid gametes from their F_1 hybrid offspring. After allowing for spontaneous hybrid diploid formation by autofertilization (*15*), we found that 81.25% were capable of sporulation and that fertility (spore viability) was high (median = 90%; mean = 84.40%, with 95% confidence interval of 73.75 to 92.67%) (Fig. 1A) (*15*). Fertility was slightly reduced from that of the parental species (*S. cerevisiae*, 99.93%, 99.04 to 99.79%; *S. paradoxus*, 99.21%, 97.80 to 99.92%) (*11*), with statistically significant variation among F_2 hybrids ($F_{61,260} = 15.72$, $P < 0.0001$). We tested for reproductive isolation of the fertile F_2 hybrids from the parental species (Fig. 1B). The backcross hybrids have fertility that is significantly higher (7.54%, 5.38 to 10.02%) than that of F_1 hybrids (0.03%, 0.00 to 0.18%) (*11*), but they have fertility that is much lower than that of the F_2 hybrids ($F_{1,895} = 817.02$, $P \ll 0.0001$). Although rare, hybrid F_2 diploids are both fertile and isolated from their parental species.

Crossing F_2 hybrids and assessing fertility of their hybrid offspring demonstrated the existence of multiple different highly fertile F_2 hybrids (*15*). Ten independent F_2 genotypes, each having 100% fertility, were randomly paired and used to generate F_3 hybrids. All pairs yielded some viable gametes, but the average fertility of F_3 hybrids (10.64%, 0.93 to 28.97%) was much lower than that of their immediate parents; also, there was genetic variation in fertility among the F_3 hybrids caused by interaction between the F_2 parental genomes ($F_{4,94} = 5.65$, $P < 0.001$). Nevertheless, autofertilized F_4 hybrid diploids derived from the viable gametes had particularly high fertility (97.33%, 92.10 to

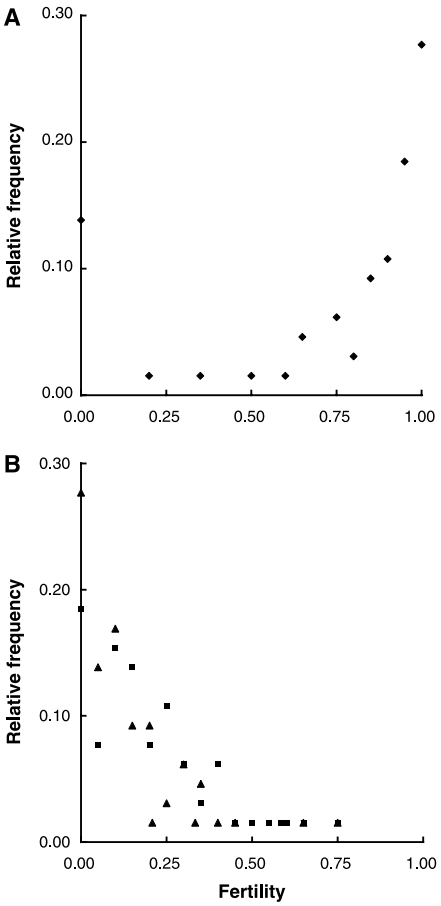

Fig. 1. Reproductive isolation of sporulation-proficient F_2 hybrids. (**A**) Hybrids have high fertility when crossed with themselves. (**B**) Hybrids have low fertility when crossed with either parental species (squares, *S. cerevisiae*; triangles, *S. paradoxus*).

[1]The Galton Laboratory, Department of Biology, University College London, Gower Street, London WC1E 6BT, UK. [2]Department of Biology, University of Houston, 4800 Calhoun Road, Houston, TX 77204, USA. [3]Department of Genetics, University of Leicester, University Road, Leicester LE1 7RH, UK.

*To whom correspondence should be addressed. E-mail: mtrav@uh.edu

99.81%), greater than that observed in the F_2 autofertilized hybrids. As in the F_2 hybrids, there was significant variation in fertility among the F_4 hybrids ($F_{13,72} = 5.02$, $P < 0.0001$), but variation in F_4 fertility was not associated with particular F_3 parental genotypes ($F_{4,13} = 0.56$, $P > 0.5$). Thus, highly fertile hybrid diploids were readily obtained regardless of existing genotype interactions.

There were several possible causes of the reproductive isolation observed between F_2 hybrids and their parents, as well as between different F_2 hybrid strains. One possibility is that crossing an F_2 hybrid with either parental genotype or another F_2 hybrid generated incompatible gene combinations in the resulting F_3 hybrids, rendering them sterile. A second possibility is chromosomal incompatibility due to the inability of chromosomes from one parental species to pair and cross over with their diverged homeologs from the other parental species (*13*). A third possible cause is aneuploidy. Meiotic segregation in F_1 hybrids is known to be ineffective, and the resulting gametes have a high frequency of disomic chromosomes (*13*). Such gametes become tetrasomic in F_2 hybrids and trisomic in backcrosses or in crosses to F_2 hybrids with different disomic chromosomes (*16*).

To distinguish between these possible causes for reproductive isolation of the F_2 hybrids, we performed two karyotype measurements on a randomly selected set of 38 hybrids. The first measurement detected the presence (or absence) of *S. cerevisiae* chromosomes through polymerase chain reaction (PCR) assays with *S. cerevisiae*–specific primers that amplify marker sequences from all *S. cerevisiae* chromosome ends, if present. This showed that 73% of the chromosomes had not recombined, and crossing over between *S. cerevisiae* and *S. paradoxus* chromosomes in the F_1 hybrid had been drastically reduced to an average of 8.1% of the *S. cerevisiae* rate. If reproductive isolation of the F_2 hybrids was due to the inability of diverged chromosomes to cross over, then strains with more *S. cerevisiae* chromosomes should be more isolated from *S. paradoxus* and less isolated from *S. cerevisiae*, and vice versa for those with more *S. paradoxus* chromosomes. This would also be expected if isolation was due to genic incompatibilities, although only if they were frequent enough to be spread across most chromosomes. No correlation between *S. cerevisiae* chromosome number and isolation from either *S. cerevisiae* ($r = 0.143$, $P > 0.2$, df = 31) or *S. paradoxus* ($r = -0.156$, $P > 0.2$, df = 31) was detected, nor was there the expected negative correlation between parental F_2 backcross fertilities ($r = -0.093$, $P > 0.5$, df = 63). Nevertheless, the power of these statistical tests is limited by the absence of fertile backcrosses and their relatively small sample sizes.

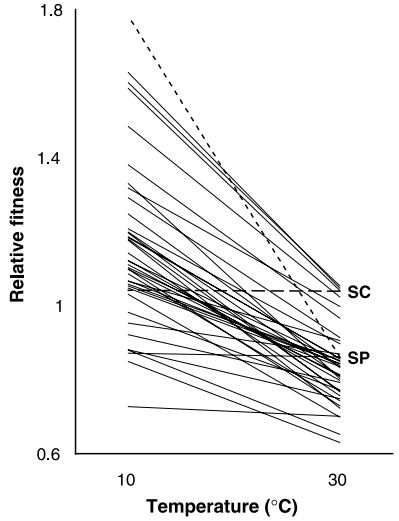

Fig. 2. Competitive ability of F_2 hybrids in two thermal environments. Hybrids have high relative fitness, in comparison with their parental species (SC, *S. cerevisiae*; SP, *S. paradoxus*), although their fitness is generally lower than that of either of the parental species at their preferred temperatures.

Instead, extensive tetrasomy was detected. *S. cerevisiae* ends were present in 797 of the 1216 individual chromosome end assays, which is 31% more frequent than that expected by chance, suggesting the presence of both *S. paradoxus* and *S. cerevisiae* homeologs of the same chromosomes in each strain. Tetrasomy was confirmed by the second karyotype assay with pulsed-field gel electrophoresis (*15*). Three *S. paradoxus* chromosomes migrate to different positions from their *S. cerevisiae* homeologs, allowing a direct and precise measure of tetrasomy. Chromosomes I, II, and VIII were tetrasomic in 26, 20, and 31% of the F_2 hybrids, respectively, consistent with the 31% overall tetrasomy estimate from the PCR assays. The abundance of tetrasomy suggests that particular chromosome combinations may have been required for viable hybrid gametes. It also provides a mechanism by which aneuploidy may play a major role in reproductive isolation of hybrids from parental genotypes, although there is no direct proof that aneuploidy is the cause.

However, neither aneuploidy nor chromosomal incompatibility adequately explains the lower self-fertility in F_2 hybrids than in the pure parental species. Unlike triploids (*16*), both nonhybrid and F_1 hybrid tetraploids are fertile (*11*), so we do not expect tetrasomes to have inherent deficiencies in meiotic segregation, although it is possible that unbalanced excess gene dosage from the extra chromosomes might affect fertility. We also can eliminate mitochondrial incompatibility (*17*) because of the high fertility of hybrid tetraploids. Chromosomal incompatibilities can be excluded because F_2 hybrids are fully homozygous, having originated from single autofertilized gametes; thus, all chromosomes can match and pair effectively. The likely explanation is that genic incompatibilities between interacting *S. cerevisiae* and *S. paradoxus* genes have a detrimental effect on fertility. The apparent absence of these incompatibilities in full tetraploid F_1 hybrids (*11*), where the complete genomes of both species are present, indicates that they are recessive. We can estimate that they contribute 50% of the variation in self-fertility among the fertile F_2 hybrids ($F_{3,52} = 6.46$, $P < 0.0001$). The nonrandom distribution of chromosomes in the F_2 hybrids suggests that similar incompatibilities also cause F_1 gamete inviability for certain combinations of chromosomes; however, the current data set is too small to determine specific interactions.

Our results suggest that homoploid hybrid speciation can occur readily and that any intrinsic incompatibilities in *Saccharomyces* can be overcome relatively easily, but extrinsic barriers, such as fitness under differing environmental conditions, could limit speciation. To address this, we compared the set of 38 F_2 hybrids and a common *S. cerevisiae* competitor in nutrient-rich medium at 30° and 10°C, temperatures that favor *S. cerevisiae* and *S. paradoxus*, respectively (Fig. 2). There was abundant genetic variation detected for fitness (*15*) among the hybrids ($F_{37,37} = 4.49$, $P < 0.0001$), and all hybrids were capable of rapid growth under both environmental conditions. Although hybrids were generally less fit than one of the parental species at each temperature, suggesting mild extrinsic incompatibility, 29% of the hybrids were more fit than *S. paradoxus* at 30°C, and 76% of the hybrids were more fit than *S. cerevisiae* at 10°C. In addition, hybrid fitness in one thermal environment was highly correlated with fitness in the other ($r = 0.786$, $P < 0.001$). Therefore, intermediate or fluctuating conditions may provide a mechanism for the selection of hybrids.

Recent studies have isolated fertile *Saccharomyces* hybrids in the laboratory (*18, 19*) and in nature (*20, 21*). In this study, we showed that homoploid hybrid speciation occurs readily in laboratory populations of *Saccharomyces*, in contrast to all known animal species and most plant species. In part, this is due to the ability to autofertilize, which produces identical homologs in every chromosome pair (except at the mating-type locus on chromosome III) and thus avoids any incompatibilities that could arise by fusion with other gametes, even from the same parent. Autofertilization is thought to be relatively common in wild yeast (*22*), and it can also occur in other species with gametophytic selfing (e.g., protists, fungi, algae, ferns). Our results extend the range of known mechanisms that cause reproductive isolation. These act at different levels and in different taxa (*23*), but all may help produce new species.

References and Notes

1. J. A. Coyne, *Nature* **355**, 511 (1992).
2. P. A. Johnson, F. C. Hoppensteadt, J. J. Smith, G. L. Bush, *Evol. Ecol.* **19**, 187 (1996).

3. H. D. Rundle, L. Nagel, J. W. Boughman, D. Schluter, *Science* **287**, 306 (2000).
4. M. White, *Modes of Speciation* (Freeman, San Francisco, 1978).
5. G. L. Bush, *Annu. Rev. Ecol. Syst.* **6**, 339 (1975).
6. E. Mayr, *Animal Species and Evolution* (Harvard Univ. Press, Cambridge, MA, 1963).
7. S. P. Otto, J. Whitton, *Annu. Rev. Genet.* **34**, 401 (2000).
8. V. Grant, *Plant Speciation* (Columbia Univ. Press, New York, 1971).
9. A. R. Templeton, *Annu. Rev. Ecol. Syst.* **12**, 23 (1981).
10. L. H. Rieseberg, *Annu. Rev. Ecol. Syst.* **28**, 359 (1997).
11. D. Greig, E. J. Louis, R. H. Borts, M. Travisano, *Proc. R. Soc. London Ser. B* **269**, 1167 (2002).
12. G. Naumov, *Stud. Mycol.* **30**, 469 (1987).
13. N. Hunter, S. R. Chambers, E. J. Louis, R. H. Borts, *EMBO J.* **15**, 1726 (1996).
14. J. A. Coyne, H. A. Orr, *Philos. Trans. R. Soc. London Ser. B* **353**, 287 (1998).
15. Materials and methods are available as supporting material on *Science* Online.
16. D. Loidl, *Genetics* **139**, 1511 (1995).
17. D. R. Taylor, C. Zeyl, E. Cooke, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 3690 (2002).
18. G. Marinoni *et al.*, *J. Bacteriol.* **181**, 6488 (1999).
19. F. Sebastiani, C. Barberio, E. Casalone, D. Cavalieri, M. Polsinelli, *Res. Microbiol.* **153**, 53 (2002).
20. I. Masneuf, J. Hansen, C. Groth, J. Piskur, D. Dubourdieu, *Appl. Environ. Microbiol.* **64**, 3887 (1998).
21. C. Groth, J. Hansen, J. Piskur, *Int. J. Syst. Bacteriol.* **49**, 1933 (1999).
22. R. K. Mortimer, *Genome Res.* **10**, 403 (2000).
23. L. H. Rieseberg, *Trends Ecol. Evol.* **16**, 351 (2001).
24. This work was supported by the Wellcome Trust.

Supporting Online Material
www.sciencemag.org/cgi/content/full/298/5599/1773/DC1
Materials and Methods
Table S1
Figs. S1 and S2
References and Notes

19 July 2002; accepted 24 September 2002

Essential Role for the SMN Complex in the Specificity of snRNP Assembly

Livio Pellizzoni,* Jeongsik Yong, Gideon Dreyfuss†

The Survival of Motor Neurons (SMN) protein, the product of the spinal muscular atrophy–determining gene, is part of a large macromolecular complex (SMN complex) that functions in the assembly of spliceosomal small nuclear ribonucleoproteins (snRNPs). Using cell extracts and purified components, we demonstrated that the SMN complex is necessary and sufficient to mediate the ATP-dependent assembly of the core of seven Sm proteins on uridine-rich, small nuclear ribonucleic acids (U snRNAs). In vitro experiments revealed strict requirements for ordered binding of the Sm proteins and the U snRNAs to the SMN complex. Importantly, the SMN complex is necessary to ensure that Sm cores assemble only on correct RNA targets and prevent their otherwise promiscuous association with other RNAs. Thus, the SMN complex functions as a specificity factor essential for the efficient assembly of Sm proteins on U snRNAs and likely protects cells from illicit, and potentially deleterious, nonspecific binding of Sm proteins to RNAs.

Nuclear pre-mRNA splicing, the process of removal of introns from pre-mRNAs, is an essential aspect of eukaryotic mRNA biogenesis that is carried out by the spliceosome. The snRNPs U1, U2, U4/U6, and U5 are essential and major components of the spliceosome. Each snRNP consists of one U snRNA molecule, a common core comprising a ring of seven Sm proteins, and several snRNP-specific proteins (*1*). The process of snRNP biogenesis, which occurs in the cytoplasm, requires the assembly of the Sm proteins on the Sm site, which is a uridine-rich sequence present in the U snRNAs, to form the Sm core (*2*). A properly assembled Sm core and the hypermethylated 5′ cap are both required to recruit the import receptors necessary for snRNP translocation into the nucleus (*1*, *3–6*). Once in the nucleus, snRNPs associate with specific proteins that are unique to each snRNA, and function in pre-mRNA splicing.

SnRNP assembly readily occurs in vitro from purified snRNP proteins and U snRNAs (*7–9*). This assembly reaction does not require ATP or non-snRNP factors. However, snRNP assembly in extracts of mammalian cells or *Xenopus laevis* eggs requires ATP hydrolysis (*10*, *11*). Considerable data reveal that a large macromolecular complex containing the Survival of Motor Neurons (SMN) protein is required for snRNP assembly (*11–14*). SMN is the product of the spinal muscular atrophy (SMA)–determining disease gene (*15*). Reduced levels of SMN protein result in SMA, a common neurodegenerative disease of the motor neurons (*16*, *17*). The SMN protein is associated with Gemin2, Gemin3, Gemin4, Gemin5, Gemin6, and Gemin7 in a large complex that localizes both in the cytoplasm and the nucleus (*18–24*). Although its role in snRNP assembly is better characterized, the SMN complex likely functions in the assembly and/or restructuring of several ribonucleoprotein particles including small nucleolar RNPs (snoRNPs) and the machineries that carry out transcription and pre-mRNA splicing (*25*). Despite advances in the characterization of the interactions and functions of the SMN complex, mechanistic insights into the molecular functions of the SMN complex in snRNP assembly have been lacking.

Using experiments in HeLa cell extracts, we showed that the SMN complex is necessary for snRNP assembly of all the major Sm site–containing U snRNAs and that this requires ATP hydrolysis (figs. S1 and S2). We therefore asked whether the SMN complex is not only necessary but also sufficient to mediate snRNP assembly. To do so, we tested the ability of purified SMN complexes to assemble Sm cores. Native SMN complexes were purified from cell lines expressing FLAG-tagged SMN or Gemin2 (*23*) by affinity chromatography on anti-FLAG beads and eluted with excess of the FLAG peptide (Fig. 1A) (*26*). SMN complexes purified from both FLAG-SMN and FLAG-Gemin2 cell lines are identical in composition and, at moderate salt concentrations, also contain Sm proteins (*23*). Purified SMN complexes were analyzed for their capacity to form Sm cores on the major Sm site U snRNAs. Through use of native gel electrophoresis under stringent conditions (*7*, *8*), we demonstrated that the SMN complex mediates snRNP assembly of Sm site–containing U snRNAs (Fig. 1B). The formation of Sm cores was confirmed by immunoprecipitation of the U snRNAs from assembly reactions with anti-Sm antibodies (*27*). ATP, but not ATP hydrolysis, is required for SMN complex–mediated Sm core assembly of U1 snRNA (Fig. 1C). These results demonstrate that a purified SMN complex containing the Sm proteins is necessary and sufficient to mediate the ATP-dependent assembly of snRNPs.

Sm core assembly can be reconstituted in vitro using purified total snRNP proteins (TPs) and in vitro–transcribed U snRNAs (*7–9*). The process of snRNP assembly with TPs is ATP-independent and does not require non-snRNP factors. We analyzed the effect of purified SMN complexes in the Sm core assembly with TPs. For these experiments, SMN complexes were purified under high salt conditions (500

Howard Hughes Medical Institute and Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104–6148, USA.

*Present address: Institute of Cell Biology, Consiglio Nazionale delle Ricerche, "Campus Adriano-Buzzati-Traverso," IBC 00016, Monterotondo Scalo, Rome, Italy.

†To whom correspondence should be addressed. E-mail: gdreyfuss@hhmi.upenn.edu