DNA preparation

For each time point to be analyzed, 3 ml aliquots of mating mixtures were removed for DNA isolation. For early zygotes or gametes, aliquots were spun down and resuspended in 0.4 mLs TEN buffer (10 mM Tris 8.0, 10 mM EDTA, 150 mM NaCl). Small scale DNA preps were then made as described below, but without the sonication, washes or zirconium beads. For later zygote DNA preps (6 hrs.+), aliquots were resuspended in 0.5 mLs TEN buffer with 0.2% NP40 in 1.5 mL tubes and sonicated (Fisher Sonic Dismembrator Model 300) on full power for 30 sec. to lyse unmated gametes. Zygotes were repelleted and washed 2-3 times more in 1 mL TEN with 0.2% NP40. After a final suspension in 0.4 mLs TEN, 40 µL 20% SDS and 40 µL 20% Sarkosyl, 0.4 mLs 1.0 mm zirconium beads (Biospec Products, Inc.) were added. The zygotes were vortexed at full power for 5 min. resulting in >99% breakage (determined microscopically). 20 µL 10 mg/mL pronase was added and samples were incubated at 37° for 30 min. 500 µL of phenol/chloroform 1:1 was added and the samples vortexed for 3 min. After a brief centrifugation, the aqueous phase was removed and precipitated with 2 volumes of EtOH. Pellets were washed with 70% EtOH, vacuum dried and resuspended in 50 µL TE.

The 5 day old zygote DNA was prepared as follows: 3 mLs of zygotes were spun down at 2 hours after mating and spread on a cellulose filter (gift of David Cove) on a HSM plate. After 24 hours in the light and 5 days in the dark, cells were scraped into 0.4 mLs TEN buffer and DNA prepared as described above.

Germinated progeny DNA was prepared by plating zygotes at 2 hours and allowing them to mature by the normal procedure (described above). After chloroform treatment to kill unmated gametes, the plates were placed in the light for 5-7 days until a lawn of germinated cells formed. Approximately $5x10^7$ progeny cells were then scraped off the plate and processed as described for gamete and early zygote DNA preparations.