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28. Supported by the Geological Survey of Namibia, NSF, Petroleum Development Oman, and Schlumberger. We thank C. Husselmann for access to Driedoornvlagte; D. McCormick and S. Schroeder for assistance in the field; D. Simons for photographic expertise; S. Conway Morris, C. Scrutton, A. Knoll, S. Bengtson, M. Brasier, M. Droser, and an anonymous reviewer for comments on the manuscript; R. Duncan-Jones and A. Brett for etymological expertise; and R. Swart (National Petroleum Corporation of Namibia) for permission to publish the LANDSAT image shown in fig. S1B.

Supporting Online Material

www.sciencemag.org/cgi/content/full/296/5577/2383/DC1

Figs. S1 and S2

Appendix S1

4 March 2002; accepted 15 May 2002

Pollen-Mediated Movement of Herbicide Resistance Between Commercial Canola Fields

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There is considerable public and scientific debate for and against genetically modified (GM) crops. One of the first GM crops, *Brassica napus* (oilseed rape or canola) is now widely grown in North America, with proposed commercial release into Australia and Europe. Among concerns of opponents to these crops are claims that pollen movement will cause unacceptable levels of gene flow from GM to non-GM crops or to related weedy species, resulting in genetic pollution of the environment. Therefore, quantifying pollen-mediated gene flow is vital for assessing the environmental impact of GM crops. This study quantifies at a landscape level the gene flow that occurs from herbicide-resistant canola crops to nearby crops not containing herbicide resistance genes.

Data on pollen dispersal has mostly been obtained from small-scale field trials of limited sample size (1–5). Canadian experiments with GM canola found less than 0.03% pollination at 30 m into conventional varieties (6). However, Hall *et al.* (7) suggested GM canola pollen moved over greater distances. Therefore, we examined pollen movement between herbicide-resistant canola and conventional varieties on a commercial scale, testing over 48 million individual plants. This was possible because canola resistant to acetolactate synthase (ALS)-inhibiting herbicides was grown commercially in Australia for the first time in 2000. These first commercial fields served as the herbicide resistance gene source in an uncontaminated environment. This variety has a two-gene

system—one herbicide resistance gene on each genome—and is homozygous for both genes. Therefore, any crosses to a conven-

tional variety will contain one copy of each gene.

To assess gene flow, seeds were collected from 63 conventional canola fields growing near herbicide-resistant fields in New South Wales, Victoria, and South Australia. These three states represent over half of the canola-growing area in Australia as well as a wide and diverse range of environments. Source and sink fields were of similar sizes, ranging from 25 to 100 ha. At crop maturity, 10 stratified samples totaling at least 100,000 seeds were taken from each of three locations in each field of conventional canola. These were parallel to the source field and taken at the edge nearest to the source field, the middle, and the edge furthest from the source field. Collected seed samples (500 g) were planted as separate plots, in an irrigated field, along with two resistant and two susceptible canola controls. To determine whether pollen-mediated gene flow from source to sink fields had occurred, we screened the seedlings with a lethal discriminating dose of the ALS-inhibiting herbicide chlorsulfuron, and any sur-

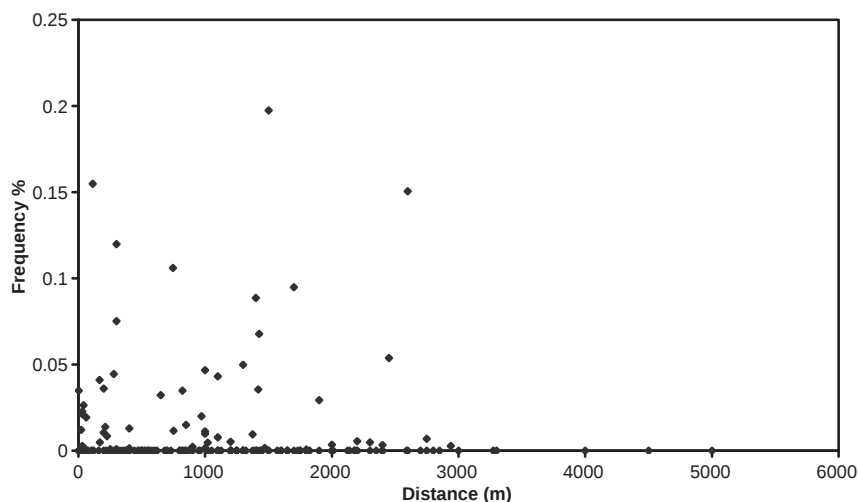


Fig. 1. Percentage of ALS herbicide-resistant individuals in seed from nonresistant varieties in relation to distance from the source field. Three individual samples were collected per field, with 190 individual collection locations.

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vivors were further confirmed by a repeat treatment 14 days later.

Only 30% of samples screened revealed ALS herbicide-resistant individuals; the remainder had no detectable resistance. Resistance frequencies varied up to a maximum of 0.197% (Fig. 1). When individual samples were pooled within fields, resistance was evident in 63% of these fields, although only a few had more than 0.03% resistance (Fig. 2). The results show that, in most cases, gene flow via pollen movement occurs between canola fields. However, even adjacent commercial canola fields in Australia will have much less than 1% gene flow. Resistance was not detected in fields more than 3 km from the source, although the number of fields sampled was not large (Fig. 2).

Previous researchers (2, 4, 6, 8) have recognized that herbicide resistance is a powerful genetic marker, providing that other sources of resistance are excluded. For ALS herbicide resistance, it is acknowledged that natural but low frequencies of resistance have been reported (9). This level was measured at 10^{-6} in the Australian variety Karoo in previous experiments conducted by our research group (data not shown). Other sources of contamination must be small, because the highest frequency of resistance detected on a field basis was 0.07% and because no resistance was detected in 23 fields, despite the examination of over 700,000 seedlings.

Edge effects have been consistently observed in all previous pollen movement work (4, 6, 10, 11), with cross-pollination occurring at higher frequencies closer to the source field. However, comparison of samples within fields did not demonstrate a consistent edge effect. That is, samples from the leading edge of fields did not always have a higher level of resistance. In fields where the front edge was less than 100 m from the herbicide-resistant field, similar frequencies of resistance were found at all three collection points within the field (Fig. 3). Overall, some fields did show a decline in resistant individuals with distance, but the majority of fields, particularly those further from the source field, were more variable. The edge effect observed in this large-scale landscape study was uniformly low and did not appear to unduly influence pollination events.

This study is unique for several reasons: it was conducted with large commercial canola fields, used large sample sizes, and was conducted over one-third of Australia, which covers a range of environments. Another unique aspect of this investigation is that the pollen sources were large (25- to 100-hectare fields), unlike other studies where relatively small sources have been used. The use of large commercial fields rather than small, artificial pollen sources has revealed that there is a small amount of pollen-mediated gene movement up to 3 km from a source field. We did not observe a leptokurtic or exponential decline, as found in

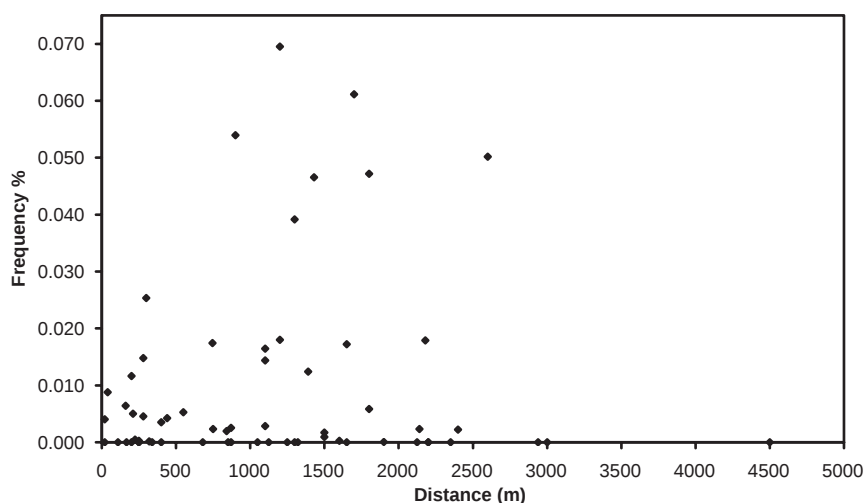


Fig. 2. Percentage of ALS herbicide-resistant individuals in seed from nonresistant varieties in relation to distance from the source field. Samples pooled per field, with 63 fields sampled.

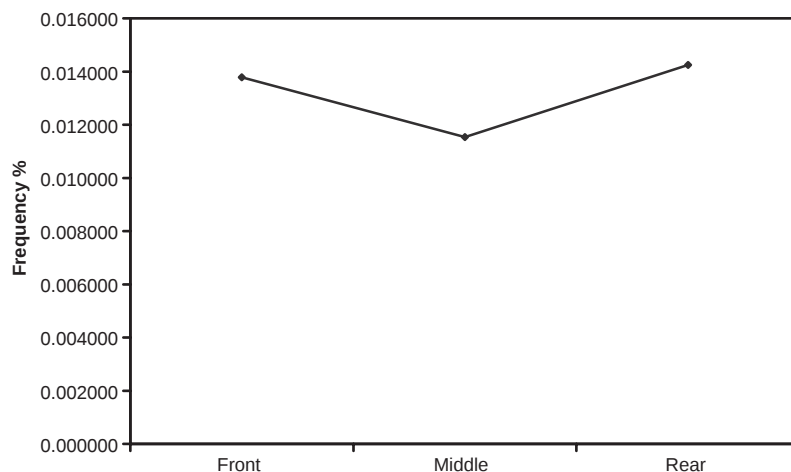


Fig. 3. Percentage of ALS herbicide-resistant individuals by location within field (edge closest to source, middle, and rear edge) of sample collection. Data are only for those fields with the nearest edge within 100 m of the pollen source.

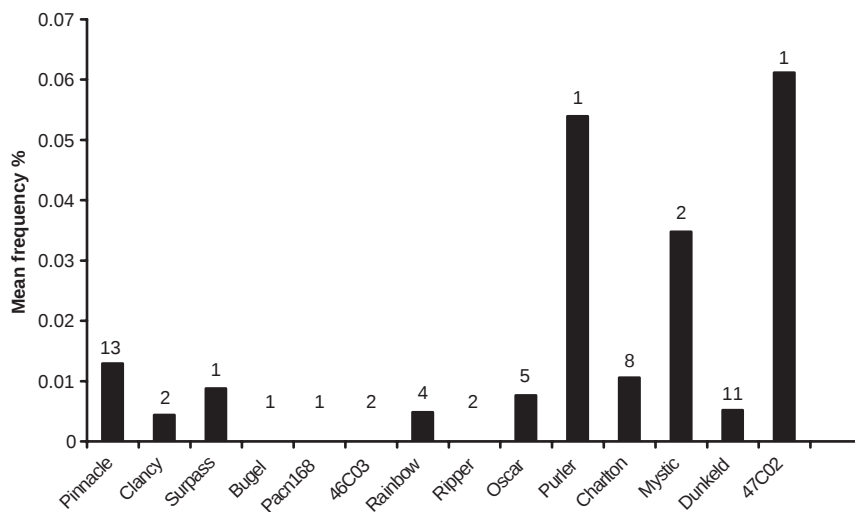


Fig. 4. Percentage of ALS herbicide-resistant individuals in sink fields by variety. The number of fields screened (numeral above each bar), percentage of resistance recorded, and variety are given.

many small-scale studies (1, 5, 10, 12). Instead, a more variable distribution with isolated pollination events was detected. The multiple pollinating agents (wind and insects) of canola and the large size of the source may contribute to the randomness of long-distance pollination events.

Varietal differences among canola sink fields were observed (Fig. 4), but no consistent effect of wind direction on pollen-mediated gene flow was detected (data not shown). The variety of canola may be a contributing factor in random pollination events at distance. Pollination has been shown to be affected by crop variety (12). Varieties have differences in flowering period, which will affect pollination events over such a large scale. Another explanation for these seemingly random events may also be related to insect behavior. Roaming insects may target single plants flowering early or late in a field, resulting in sporadic pollen movement. However, insects are more likely to remain in a single field if sufficient resources (e.g., flowers) are readily available (13).

Gene transfer is a complex process and is dependent on many factors (14–16), including environmental conditions, plant variety, insect behavior, and plant density. These observations, coupled with our data on long-distance pollen movement, indicate that laboratory and small-scale experiments may not necessarily predict pollination under commercial conditions. This study demonstrates that cross-pollination between commercial canola fields occurs at low frequencies but to considerable distance.

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8 March 2002; accepted 13 May 2002

Regulation of Hypoxic Death in *C. elegans* by the Insulin/IGF Receptor Homolog DAF-2

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To identify genetic determinants of hypoxic cell death, we screened for hypoxia-resistant (Hyp) mutants in *Caenorhabditis elegans* and found that specific reduction-of-function (rf) mutants of *daf-2*, an insulin/insulinlike growth factor (IGF) receptor (INR) homolog gene, were profoundly Hyp. The hypoxia resistance was acutely inducible just before hypoxic exposure and was mediated through an AKT-1/PDK-1/forkhead transcription factor pathway overlapping with but distinct from signaling pathways regulating life-span and stress resistance. Selective neuronal and muscle expression of *daf-2(+)* restored hypoxic death, and *daf-2(rf)* prevented hypoxia-induced muscle and neuronal cell death, which demonstrates a potential for INR modulation in prophylaxis against hypoxic injury of neurons and myocytes.

Although genetically tractable model organisms have made longstanding contributions to our understanding of programmed cell death (1) and recently to identification of molecular mechanisms of hypoxic adaptation and sensing (2, 3), direct genetic screens for hypoxia-resistant mutants have been relatively unexplored. To identify genes that regulate hypoxic cell death, we screened new and existing mutant strains for animals that survived exposure to either hypoxia or sodium azide (4), an electron-transport chain inhibitor used as a chemical surrogate for hypoxia. High-level resistance to hypoxia or azide was an uncommon phenotype. We identified only two new mutants and a few existing ones that had significantly improved survival. We found the strongest Hyp strains among existing mutants with reduced activity of the insulin/IGF receptor (INR) signaling pathway. *daf-2(e1370)*, which carries a rf mutation in the homolog of the human insulin/IGF receptor (5), was markedly azide resistant compared with wild-type strain N2 ($13.2 \pm 1.8\%$ dead versus $80.8 \pm 5.9\%$; $P < 0.0001$). Subsequent hypoxic incubation demonstrated that *daf-2(e1370)* was indeed Hyp (Fig. 1, Table 1). Genetic mapping confirmed the *e1370* mutation was responsible for the Hyp phenotype (4). *daf-2(e1370)* not only survived but fully

recovered normal locomotion behavior after as long as 20 hours of hypoxic incubation (Fig. 1A, movies S1 and S2). N2 displayed significant locomotion defects after recovery from a 6.5-hour incubation. Hypoxic sensitivity was not stage or age specific with the exception of N2 dauers (a long-lived alternative larval stage), which were Hyp (Fig. 1C). The Hyp phenotype of *daf-2(e1370)* was markedly sensitive to temperature; *e1370* animals were less

Table 1. *daf-2* allelic variation for hypoxia resistance (Hyp). Animals were raised at 20°C except *sa187*, *e1369*, *e979*, which were raised at 15°C then shifted to 20°C 2 days before testing. Percent dead is reported as means $\pm$ SEM per trial. Adults 2 days post L4 were exposed to <0.3% oxygen at 28°C for 20 hours then scored after a 24-hour recovery period. Each trial was a completely independent experiment done on a different day.

Genotype	Percent dead	Trials (n)	Animals (n)
+/+	95.5 $\pm$ 1.2	26	2568
<i>daf-2(e1370)</i>	3.6 $\pm$ 1.3*	26	835
<i>daf-2(sa219)</i>	4.6 $\pm$ 2.4*	4	699
<i>daf-2(m579)</i>	8.6 $\pm$ 3.8*	5	110
<i>daf-2(e1369)</i>	46.4 $\pm$ 15.6*	4	185
<i>daf-2(m596)</i>	47.0 $\pm$ 3.6*	4	210
<i>daf-2(sa187)</i>	53.3 $\pm$ 6.3*	5	318
<i>daf-2(e979)</i>	55.6 $\pm$ 12.4*	4	330
<i>daf-2(e1391)</i>	59.0 $\pm$ 11.7*	5	174
<i>daf-2(e1368)</i>	77.3 $\pm$ 7.7	4	344
<i>daf-2(sa229)</i>	80.1 $\pm$ 10.6	6	547
<i>daf-2(e1365)</i>	87.9 $\pm$ 6.7	4	330
<i>daf-2(m577)</i>	90.9 $\pm$ 6.7	3	199
<i>daf-2(e1371)</i>	95.8 $\pm$ 2.4	4	292

* $P < 0.01$ versus wild type by Mann-Whitney test.

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