Mutation Rates and Dominance Levels of Genes Affecting Total Fitness in Two Angiosperm Species

Mark O. Johnston* and Daniel J. Schoen

Theories about the evolution of sex and the effects of inbreeding depend on knowledge of the mutation rate and dominance level of deleterious alleles affecting total fitness. In two species of largely self-fertilizing annual plants, minimal estimates of such mutation rates were found to be 0.24 to 0.87 per sporophyte genome per generation, but confidence intervals exceeded 1.0 in each of the four populations. Dominance levels were near zero in one species and intermediate (0.28 to 0.35) in the other. These results suggest that the detrimental effects of inbreeding are a result of new partially recessive mutations rather than overdominance.

The mutation rate and dominance level of deleterious alleles are decisive elements in genetic theories of the evolution of sex (*1*) and recombination (*2*) and the coevolution of inbreeding depression and rate of self-fertilization (*3–5*). The deterministic mutation hypothesis for the evolution of sex, for example, requires that mutation rates exceed one per genome per generation (*6*). The generality of such theories can be determined only with data from a wide spectrum of phylogenies and mating systems. The few estimates of mutation and dominance that have been made are primarily for viability in the fruit fly *Drosophila* (*7*), and none is for total fitness in a natural population (*6*). Annual plant populations that are largely self-fertilizing provide an opportunity to estimate both dominance levels and mutation rates for genes affecting total fitness in natural populations. Total lifetime fitness can be estimated by the number of seeds produced autonomously; the usual difficulties of estimating the male-fertility component achieved through pollen dispersal do not exist. And, because such populations already consist of completely or nearly completely homozygous lines, complications arising from artificial establishment of inbred lines (*7*) are avoided. Progeny fitnesses from self- and outcross fertilizations of homozygous individuals yield estimates of inbreeding depression (heterosis), dominance levels of deleterious mutations (*7, 8*), and mutation rates (*9*). Here we present evidence that inbreeding depression in lifetime fitness for two self-fertilizing angiosperms is caused by incompletely recessive alleles arising through mutation at a rate similar to that reported for *Drosophila* (*10*).

The deleterious effects of close inbreeding have been well documented in both agricultural and natural species of plants and animals (*11, 12*). In plants, inbreeding depression is the proportional reduction in the fitness of progeny from self-fertilization (selfing) as compared with that of progeny from outcrossing. Two primary genetic causes have been proposed, differing in the dominance of causative alleles but both depending on the fact that selfing increases offspring homozygosity (*13*). The overdominance hypothesis suggests that the heterozygote is superior to both the homozygous dominant and the homozygous recessive. The partial dominance hypothesis suggests rather that inbreeding depression is caused by exposure in the homozygous state of wholly or incompletely recessive deleterious alleles newly arisen through mutation. If the fitnesses at a single locus of nonmutant and mutant homozygotes are 1 and $1 - s$, respectively, then the heterozygote fitness is $1 - hs$, where h represents the level of dominance, or the degree to which the deleterious allele is expressed in the heterozygote (*14*). Negative values of h represent overdominance, those between 0 and 0.5 represent partial dominance (primarily recessive alleles), and a value of 0.5 indicates additivity. A value less than 0.5 signifies inbreeding depression.

If caused by partial dominance of deleterious mutations, inbreeding depression should decrease with the self-fertilization rate in natural populations at equilibrium, because deleterious mutations are exposed to selection in the homozygous state (*3, 4*). Under a model of multiplicative fitness effects of mutations, the extent of inbreeding depression in a wholly self-fertilizing population is determined by the dominance coefficient and the mutation rate per diploid genome per generation, U: $\delta \approx 1 - \exp - (1/2 - h)U$, and not by the selection coefficient, s, an approximation that also holds for a model of synergistic epistasis and selfing rates as low as 0.9 (*15*). Thus, higher mutation rates and lower dominance coefficients cause a greater amount of inbreeding depression in a population at equilibrium.

Amsinckia (Boraginaceae) is a genus of annual plants in which self-fertilization has evolved at least four times from still-extant outcrossing species or their common ancestors (*16*). In contrast to their more highly outcrossing progenitors, plants in highly selfing populations have smaller flowers, lack heterostyly, and set seed autonomously. We studied two populations in California of each of two species, *Amsinckia gloriosa*, a tetraploid weed with disomic inheritance, and *A. spectabilis* var. *spectabilis*, a diploid confined to coastal bluffs. For each population, the natural rate of self-fertilization was estimated from field-collected seeds (*17*), and a mating design was used to estimate inbreeding depression, dominance

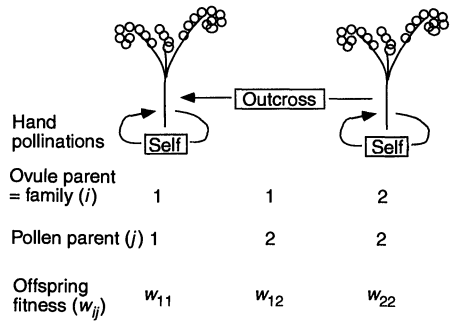

Fig. 1. Experimental design. Arrows indicate the direction of manual pollen transfer in producing the selfed and outcrossed progeny, for which the fitness measures are w_{ii} and w_{ij}, respectively, where $i \neq j$. Naturally produced seeds collected from the four California populations were germinated at the McGill University phytotron. Some of these were subjected to electrophoresis to estimate the selfing rate (Table 1), whereas others were grown to flowering and used in the controlled matings. Flowers were emasculated before anther dehiscence, and on each plant, two were hand-selfed and two were hand-outcrossed with the use of pollen from one other plant. For each family, one resulting selfed and outcrossed seed was used to evaluate four sequential and nonoverlapping components of fitness: germination, survival to flowering, flower number of survivors, and autonomous seed production. Fitness measured as germination through flowering was the number of flowers produced by a seed and thus encompasses survival of a seed to flowering as well as flower number. The total fitness of an individual selfed or outcrossed offspring (seed) was the number of seeds it produced autonomously. Germination and survival to flowering were each nearly 100% for both treatments and are not reported. Inbreeding depression for any trait was $1 - \mu(w_{ij})/\mu(w_{ij})$ where μ indicates the mean, and dominance was the coefficient from the regression of w_{ij} on $w_{ii} + w_{jj}$.

M. O. Johnston, Department of Biology, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, B3H 4J1, Canada.
D. J. Schoen, Department of Biology, McGill University, 1205 Avenue Docteur Penfield, Montreal, Quebec, H3A 1B1, Canada.
*To whom correspondence should be addressed.

levels, and mutation rates (*18*) (Fig. 1). Selfing rates exceeded 99% in all four populations (Table 1). Inbreeding depression in total fitness, measured as the number of seeds produced by a seed, ranged from 9.9% (indistinguishable from zero) to 17% ($P \ll 0.001$; Table 2).

Mutation rates per sporophyte genome for genes affecting total fitness were 0.87 and 0.79 in *A. gloriosa* populations and 0.40 and 0.24 in *A. spectabilis* (Table 2). Confidence intervals indicated that these rates are greater than zero for *A. gloriosa* and possibly greater than one in all populations. Mutations affecting total fitness were significantly nonadditive ($h < 0.5$) in three of the four populations (Table 2). Dominance coefficients in both *A. spectabilis* populations were indistinguishable from zero, indicating recessivity, whereas those in *A. gloriosa* exhibited incomplete recessivity (partial dominance, $h > 0$; Table 2). Mutations affecting flower number itself showed consistent values of partial dominance across the four populations, ranging from 0.23 to 0.32 (Table 2). Although the only fitness component exhibiting overdominance was autonomous seed production in the Alisal Slough population of *A. spectabilis*, the confidence intervals indicated that some role for overdominant mutations affecting total fitness could not be ruled out in either population of this species.

Mutations decrease the average fitness of populations by a proportion defined as the mutational load. Compared with otherwise identical populations with no mutations, the present study suggests that population mean fitnesses are decreased by about 35 and 33% in the two *A. gloriosa* populations and by 18 and 11% in *A. spectabilis* ($1 - \exp - U/2$) (*15*). At equilibrium, a higher selfing rate increases the number of homozygous mutations per individual but decreases the heterozygous number to a greater extent, causing a decrease in the load (*4*, *19*). Under a multiplicative selection model, the load in random-mating populations is one to two times the mutation rate, depending on whether the mutations are wholly or partially recessive (*7*, *14*, *20*). Thus, mutational loads in corresponding wholly outcrossing populations would be 87 and 79% in *A. gloriosa* (= 2 × rate per gamete for $h > 0$) and 20 and 12% in *A. spectabilis* (= rate per gamete, assuming $h = 0$) (*14*). Compared

Table 1. Estimated rates of self-fertilization in the four populations of *Amsinckia*.

Species and popluation	Selfing rate (S)	95% confidence interval	Number of families	Number of offspring	Isozyme marker
		A. gloriosa			
Paloma Creek Canyon	0.999	0.999, 0.999	45	256	Aat
New Idria	0.997	0.994, 0.999	69	396	Idh
		A. spectabilis			
Alisal Slough	0.998	0.781, 0.999	49	291	Adh
Zumdowski State Beach	1.6	−1.2, 4.5	53	159	(see text)

Table 2. Estimated inbreeding depression (δ), dominance levels (*h*), mutation rates per sporophyte genome per generation (*U*) of genes affecting fitness components as well as total fitness in the four populations of *Amsinckia*. CI, confidence interval.

Fitness measure	Inbreeding depression			Dominance			Mutation rate	
	δ	*P*	Number of families	*h*	95% CI	Number of families	*U*	95% CI
				A. gloriosa: *Paloma Creek Canyon*				
Flower number of survivors	0.049	0.049	95	0.23	0.14, 0.31	92		
Fitness: germination through flowering	0.091	0.002	100	0.32	0.25, 0.40	100		
Autonomous seed production	0.11	0.002	95	0.25	0.16, 0.34	92		
Total fitness	0.17	<0.001	85	0.28	0.15, 0.43	85	0.87	0.49, 2.57
				A. gloriosa: *New Idria*				
Flower number of survivors	0.042	0.152	80	0.32	0.17, 0.50	73		
Fitness: germination through flowering	0.016	0.36	88	0.27	0.12, 0.47	84		
Autonomous seed production	0.13	<0.001	78	0.26	0.11, 0.57	68		
Total fitness	0.11	0.003	67	0.35	0.26, 0.47	67	0.79	0.17, 2.70
				A. spectabilis: *Alisal Slough*				
Flower number of survivors	0.022	0.35	63	0.32	0.12, 0.51	55		
Fitness: germination through flowering	−0.019	0.58	78	0.41	0.27, 0.55	75		
Autonomous seed production	0.038	0.23	45	−0.20	−0.49, −0.047	27		
Total fitness	0.14	0.007	24	0.14	−0.32, 0.59	24	0.40	−1.17, 2.89
				A. spectabilis: *Zmudowski State Beach*				
Flower number of survivors	0.040	0.019	97	0.31	0.14, 0.53	91		
Fitness: germination through flowering	−0.016	1	104	0.45	0.25, 0.67	100		
Autonomous seed production	0.018	0.30	58	0.078	−0.29, 0.28	35		
Total fitness	0.099	0.10	35	0.069	−0.25, 0.37	35	0.24	−0.16, 1.11

with multiplicativity, synergistic selection would increase inbreeding depression but greatly decrease mutational load (*7*, *15*).

Although some data both from agricultural plant species and from *Drosophila* suggest overdominance, evidence indicates that detrimental recessive alleles are of overwhelming importance in causing inbreeding depression (*11*, *12*, *21*). The conclusions from previous plant studies, however, are often complicated by artificial selection on characters whose relation to fitness in a natural environment is unknown (*22*). One study of a natural population analyzed linked marker genes and found evidence for a range of dominance, including $0.5 < h < 1$ (*23*). The dominance data from *Drosophila* are limited to parts of the life cycle, rather than total fitness, and suggest levels of about 0.02 for lethal and 0.2 to 0.4 for mildly detrimental viability mutations (*6*, *8*).

There are fewer estimates of deleterious mutation rates. In plants, estimates for chlorophyll-deficient lethals range from 3.1×10^{-4} to 1.4×10^{-3} per diploid genome per generation in several annuals with various mating systems (*24*, *25*). A much higher rate, 7.4×10^{-3} per haploid genome per reproductive episode, was found in the long-lived, highly selfing red mangrove *Rhizophora mangle* (*25*). In laboratory strains of *Drosophila*, rates per diploid genome per generation are approximately 0.02 for lethals and 1.0 for mild detrimentals affecting viability alone (*6*, *21*). In a study of mutations affecting both viability and fertility (total fitness) in *Drosophila*, the rate was estimated at 0.5 for total fitness as well as for viability alone, which suggests that viability mutations act pleiotropically to decrease fertility as well (*10*). In *Amsinckia*, in contrast, statistically significant inbreeding depression was often detectable in fertility components and life stages including fertility components but not in viability of the vegetative stage (the period from seed maturation to flowering).

Our analysis assumes that the populations are in mutation-selection equilibrium. Inbreeding depression could be low in any of these populations as a result of loss of deleterious alleles during recent bottlenecks. This was a primary motivation for conducting the analyses in more than one population. Nevertheless, although the original seeds were from natural populations, the estimations of progeny fitness were conducted in relatively noncompetitive conditions with ample resources, so that the fitness of selfed progeny could be greater than under natural conditions (*26*). For example, if the ratio of selfed to outcrossed progeny fitness was overestimated by a factor of 2, inbreeding depression in the four populations would be 0.59, 0.56, 0.57, and 0.55, and the calculated mutation rates, assuming the same h, would be 4.0, 5.4, 2.3, and 1.9. The estimation of dominance assumes a multiplicative selection model. If mutations decreased fitness according to synergistic epistasis, h would be slightly underestimated, again leading to underestimation of U. The results from this study, therefore, must indicate minimum estimates of the mutation rate. Whether multiplicativity or synergism is the correct selection model in natural populations, these estimates are sufficiently high for mutation to be a major force in the evolution of reproductive systems (*1*, *4*, *6*, *15*). It remains to be discovered whether degrees of pleiotropy for viability and fertility genes differ between plants and animals and whether genomic mutation rates are constant among species differing in genome size, as has been suggested for some microbes (*27*).

REFERENCES AND NOTES

1. A. S. Kondrashov, *Genetics* **111**, 635 (1985).
2. B. Charlesworth, *Genet. Res.* **44**, 199 (1990).
3. R. Lande and D. W. Schemske, *Evolution* **39**, 24 (1985).
4. D. Charlesworth, M. T. Morgan, B. Charlesworth, *ibid.* **44**, 1469 (1990).
5. M. K. Uyenoyama and D. M. Waller, *Theor. Popul. Biol.* **40**, 14 (1991).
6. A. S. Kondrashov, *Nature* **336**, 435 (1988).
7. M. J. Simmons and J. F. Crow, *Annu. Rev. Genet.* **11**, 49 (1977); J. F. Crow and M. J. Simmons, in *The Genetics and Biology of* Drosophila, M. Ashburner, H. L. Carson, J. N. Thompson, Eds. (Academic Press, New York, 1983), vol. 3c, pp. 1–35.
8. T. Mukai, S. I. Chigusa, L. E. Mettler, J. F. Crow, *Genetics* **72**, 335 (1972).
9. B. Charlesworth, D. Charlesworth, M. T. Morgan, *Nature* **347**, 380 (1990).
10. D. Houle, D. K. Hoffmaster, S. Assimacopoulos, B. Charlesworth, *ibid.* **359**, 58 (1992).
11. S. Wright, *Evolution and the Genetics of Populations*, vol. 3, *Experimental Results and Evolutionary Deductions* (Univ. of Chicago Press, Chicago, 1977).
12. D. Charlesworth and B. Charlesworth, *Annu. Rev. Ecol. Syst.* **18**, 237 (1987).
13. J. F. Crow, in *Heterosis*, J. W. Gowen, Ed. (Iowa State College Press, Ames, IA, 1952), pp. 282–297.
14. ———, in *Mathematical Models in Population Genetics*, K.-I. Kojima, Ed. (Springer, Berlin, 1970), pp. 128–177.
15. B. Charlesworth, M. T. Morgan, D. Charlesworth, *Genet. Res.* **57**, 177 (1991).
16. P. M. Ray and H. F. Chisaki, *Am. J. Bot.* **44**, 537 (1957); D. J. Schoen, A.-M. L'Heureux, J. M. Marsolais, M. O. Johnston, unpublished results.
17. A screening of 11 isozymes by means of starch-gel electrophoresis yielded one polymorphic Mendelian marker in three of the four populations. To estimate the selfing rates in these populations, genotypes of approximately six offspring per family (the maternal parent) were submitted to the MLT program [K. Ritland, *J. Hered.* **81**, 235 (1990)]. Confidence intervals (95%) were the 2.5 and 97.5 percentile points of 1000 bootstraps, with resampling occurring within families. The assumption of very high homozygosity in calculation of dominance levels and mutation rates is supported by the lack of variation at 10 of 11 markers assayed in all four populations; the selfing rate indistinguishable from one; and the fact that in the three populations with a variable marker locus, the proportion of homozygous parents was 1.0, 0.96, and 0.96, in the order presented in Table 1. Because no polymorphic marker was available for the Zmudowski State Beach population, the selfing rate was estimated by the inbreeding-depression method, in which $S = (w_x - w_n)/(w_x - w_s)$, where w_x, w_n, and w_s are the mean fitnesses of progeny from hand outcrossing, natural pollination, and hand self-fertilization, respectively (*12*). The selfing rate was estimated with the use of the period from germination through autonomous seed production as the fitness measure. The confidence interval was determined by the method of D. Charlesworth [*Heredity* **61**, 469 (1988)] (with the sign preceding the penultimate covariance term corrected to "+" in the variance formula). This estimate should not be viewed as exact, because the naturally produced seeds were produced in the field, being siblings of the parent to the selfed and outcrossed plants, which were from the phytotron. The estimate exceeded 100% because the value for naturally produced offspring was lower than that from selfing. Further evidence of a selfing rate near 100% in this population was provided by lack of a significant difference between selfed and naturally produced progeny.
18. Under the multiplicative fitness model, the effect of each additional mutation is constant and is independent of the number of existing mutations. A cross between two individuals homozygous for n_i and n_j different mutations produces offspring heterozygous for these mutations at $(n_i + n_j)$ loci, with fitness $(1 - hs)^{(n_i + n_j)}$ (*15*). The approximate average level of dominance, h, was estimated by the slope of the regression of outcrossed-progeny fitness on the sum of the selfed-progeny fitnesses from the parents constituting the outcross mating (Fig. 1) (*7*, *8*). The crosses also provided measures of inbreeding depression, $\delta = 1 - w_s/w_x$, where w_s and w_x are the mean phenotypes (fitness measures) of the products of selfing and outcrossing, respectively (Fig. 1). One-tailed significance levels of δ were determined by the proportion of 1000 bootstrap results less than zero. In each case, these agreed with Wilcoxon's signed-rank test for paired data. Mutation rates for total fitness were calculated as $U \approx -2 \ln (1 - \delta)/(1 - 2h)$ (*9*). Confidence intervals for h and U were obtained with the use of the bias-corrected and accelerated (BC_a) method of bootstrap [B. Efron and R. J. Tibshirani, *An Introduction to the Bootstrap* (Chapman and Hall, New York, 1993)], as follows: For each trait in all populations, family data consisting of fitness of the offspring from cross-fertilization as well as from self-fertilization of the two crossed parents were resampled with replacement to construct 1000 bootstrap data sets, each having the original sample size. For each bootstrap data set, h (and U in the case of total fitness) was estimated as described above. Because our calculations are appropriate for mildly deleterious mutations rather than lethals, we, following Mukai *et al.* (*8*), excluded plants with total-fitness values of zero from the estimation of h and U. Proper estimation of U and its confidence interval further required that we restrict our estimation of inbreeding depression in total fitness to the same families. [Use of all available families gave similar estimates of δ: 0.18 (n = 94), 0.12 (n = 77), 0.11 (n = 45), and 0.061 (n = 58) in the order presented in Table 2.] Levels of inbreeding depression in the other fitness components required only the progeny from self- and cross-fertilization of the maternal parent, hence the larger sample sizes for the estimates of δ than for h in Table 2.
19. T. Ohta and C. C. Cockerham, *Genet. Res.* **23**, 191 (1974).
20. J. B. S. Haldane, *Am. Nat.* **71**, 337 (1937).
21. J. F. Crow, *Oxf. Surv. Evol. Biol.* **9**, 3 (1993).
22. J. L. Jinks, in *Heterosis: Reappraisal of Theory and Practice*, R. Frankel, Ed. (Springer-Verlag, New York, 1983), pp. 1–46; G. F. Sprague, *ibid.*, pp. 47–70.
23. Y.-B. Fu and K. Ritland, *Genetics* **136**, 323 (1994).
24. D. W. Crumpacker, *Evol. Biol.* **1**, 306 (1967); O. Ohnishi, *Jpn. J. Genet.* **57**, 623 (1982); J. H. Jorgensen and H. P. Jensen, *Hereditas* **105**, 71 (1986); J. H. Willis, *Heredity* **69**, 562 (1992).
25. E. J. Klekowski Jr. and P. J. Godfrey, *Nature* **340**, 389 (1989).
26. J. Schmitt and D. W. Ehrhardt, *Evolution* **44**, 269 (1990); M. R. Dudash, *ibid.*, p. 1129; M. O.

Johnston, *ibid.* **46**, 688 (1992); L. M. Wolfe, *ibid.* **47**, 374 (1993).
27. J. W. Drake, *Proc. Natl. Acad. Sci. U.S.A.* **88**, 7160 (1991).
28. We are grateful to K. Barton, N. Wong, J. Crocker, and especially A.-M. L'Heureux for help in the laboratory and phytotron; to C. Cooney and M. Romer for phytotron expertise; to F. R. Ganders for his key to *Amsinckia*; to anonymous reviewers for comments on the manuscript; and to J. H. Willis for perspicacious discussion. Supported by grants from NSERC.

13 July 1994; accepted 14 November 1994

Inhibition of Host Gene Expression Associated with Plant Virus Replication

Daowen Wang and Andrew J. Maule*

Pea seed–borne mosaic virus (PSbMV) RNA replication in pea cotyledonary tissues was restricted largely to a zone of cells close to the infection front. In situ hybridization probes representing nine genes from two pathways of metabolism failed to detect RNA transcripts within this zone, although transcripts were found in similar amounts in tissues on either side of the zone. Thus, in common with some animal viruses, PSbMV transiently suppresses the expression of host genes. Host protein accumulation was also affected. These observations provide insights into virus-plant interactions and symptom expression.

Viruses exert their negative effects by influencing the metabolism of the host (*1*, *2*), but the mechanism is poorly understood for plant viruses. Although alterations in plant gene expression have been linked to viral infection, they have not been related to specific events in virus multiplication (*3*). We used here a histochemical approach to identify cells in which virus is actively replicating and to study the associated activity of host genes.

Pea seed–borne mosaic virus (PSbMV) is a member of the potyviridae (*4*) and has a positive-sense RNA genome of approximately 10 kilobases (kb). It is transmitted in seeds after infection of the embryo early in development (*5*). As the infection proceeds, the virus invades the cotyledon on an advancing front (*6*).

To identify cells supporting active virus replication, we probed sections of immature infected pea embryos for virus coat protein with a PSbMV antibody, and for the positive and negative senses of the RNA genome with the use of in situ hybridization (*7*). Detection of both coat protein and positive PSbMV RNA showed that the virus had accumulated in tissues proximal to the contact point with the embryonic axis, leaving a distal portion of cotyledon uninfected (Fig. 1, B and C). In the infected area, there was a uniform accumulation of virus with a sharp differentiation between infected and uninfected areas, which suggests that PSbMV replication is rapid. Estimates for the rate of advancement of infections by a related potyvirus, tobacco etch virus, suggest that the virus infects approximately one new cell every 2 hours (*8*).

Negative sense RNA is part of the replicative form of the virus (*9*). Negative sense RNA is mostly single-stranded in tissues involved in active viral RNA replication but is predominantly part of a duplex when RNA replication is completed (*10*). In situ hybridization to detect PSbMV negative sense RNA identified a zone of tissue along the periphery of the infected area where this RNA was most abundant, with much smaller amounts detected within the infected area (Fig. 1D). Hence, cells in this peripheral zone were those most recently involved in active viral RNA replication. We could not distinguish whether the weak signal seen farthest from the infection front was due to degradation of the replicative form of viral RNA or to our inability to denature fully double-stranded RNA.

To investigate the consequence of virus replication on the metabolism of the host, we used the same approach to analyze the expression of nine pea genes in tissues of the cotyledon. Five genes encoded the pea seed storage proteins legumin (three members of one gene family) (*11*), vicilin (*12*), and convicilin (*13*). Four genes encoded isoforms of granule-bound starch synthase (GBSSI and GBSSII) (*14*) and the large and small subunits of adenosine diphosphate glucose pyrophosphorylase (ADPGP) (*15*), all of which are enzymes involved in starch biosynthesis. These genes represented two pathways of metabolism, different steady-state amounts of transcripts, and different timing of expression in relation to embryo development (*14*). Sections derived from uninfected and PSbMV-infected embryos were treated with strand-specific probes for PSbMV RNA or for transcripts of the nine host genes (*16*). In sections of healthy tissue, host transcripts showed uniform distribution in the cortical tissue of the cotyledon, with greater expression of the seed storage protein genes in the peripheral and surface layers of the cotyledon (Fig. 2). In contrast, in sections cut from infected embryos, a band of cotyledonary cells with barely detectable host gene transcripts was consistently seen. This band was seen regardless of whether the transcripts were normally found in large (for example, convicilin) (6) or small (for example, ADPGP) (6) amounts. In the cells that had been infected earlier and in which the amount of PSbMV negative-sense RNA was declining, transcripts of the nine host genes accumulated to an extent comparable to that found in uninfected cells of the same cotyledon section (Fig. 2, D through L). From the relative positions of this transcript-deficient band, the PSbMV-infected area, and the periph-

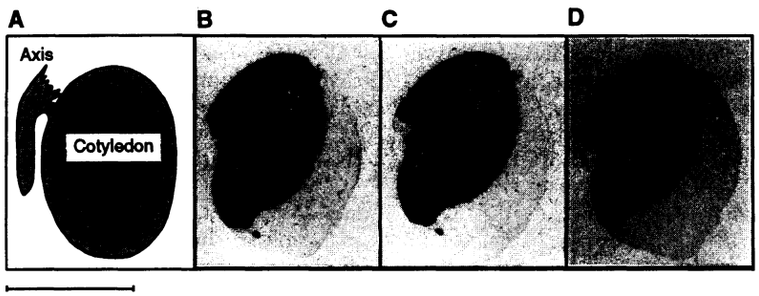

Fig. 1. Localization of PSbMV RNA and capsid protein in infected pea cotyledons. A PSbMV-infected pea embryo was sectioned close to the union between the embryonic axis and the cotyledon as shown in (**A**), and serial sections were probed for the location of virus-specific protein and RNA. Virus capsid protein (**B**) was detected by immunohistochemistry with a monoclonal antibody, whereas virus positive- (**C**) and negative- (**D**) sense RNA were detected by in situ hybridization (*7*). There was a correlation between the distribution of viral capsid protein and the genomic positive-sense RNA and a sharp boundary between the areas of infected and uninfected tissue. The faint spots of signal seen in the uninfected area result from loosened cells becoming displaced to distant areas during sectioning and processing. Viral negative-sense RNA accumulation was greatest close to the infection boundary, indicating a narrow zone of active viral RNA replication (arrow). Size bar: 5 mm.

Department of Virus Research, John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK.

*To whom correspondence should be addressed.