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Localization of *nod-3*, a Gene Conditioning Hypernodulation, and Identification of a Novel Translocation in *Pisum sativum* L. cv. Rondo

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The gene *nod-3*, which conditions hypernodulation in pea (*Pisum sativum* L.), was localized to linkage group I by linkage to markers *d* (anthocyanin ring at the base of stipules) and *Idh* (isocitrate dehydrogenase). This region of the pea genome has an unusual concentration of genes involved in the legume-*Rhizobium* symbiosis. Line Nod 3 and its parent cv. Rondo have a novel translocation which was characterized cytogenetically. The translocation does not effect segregation of the genes *nod-3*, *d* and *Idh*. One of its breakpoints is situated near gene *b* (pink flowers) of linkage group III, which corresponds to chromosome 5. The second partner of the translocation remains to be determined.

Legume mutants with an altered symbiosis provide useful material for studying the role of the plant in establishing effective nitrogen fixation. In pea, about 30 nonallelic *nod* or *sym* genes have been identified. Some genes, conferring strain specificity, were found as naturally occurring variants among primitive cultivars from the Middle East (Kneen and LaRue 1984; Lie 1984). However, most of the genes have been identified in symbiotic mutants induced in commercial cultivars of *Pisum sativum* (Duc and Messager 1989; Jacobsen 1984; Kneen and LaRue 1988). Compilations of these genes are presented in the reviews by LaRue and Weeden (1992) and Brewin et al. (1993).

Although most induced mutants have few or no nodules, some have excessive nodulation. Nine such mutants in soybean are alleles of a single gene *nts* (Delves et al. 1988). Among six hypernodulating mutants in pea—nod-3 (Jacobsen and Feenstra 1984), 190F, 191F, P77, P79 (Duc and Messager 1989), and k301 (Sidorova and Uzhintseva 1992)—two (190F and 191F) have been found to be allelic (Duc and Messager 1989), but other allelism tests have not been made, and the number of complementation groups remains uncertain. The two mutant lines Nod 3 and 190F exhibit considerably different phenotypes, and corresponding genes are tentatively treated as different loci (LaRue and Weeden 1992). In the absence of a thorough complementation analysis, the mapping of the mutants can partially answer the question of how many genes are affected by the hypernodulating mutations and where they map in relation to other genes involved in symbiotic nitrogen fixation.

A not uncommon problem with mapping studies in pea is appearance of unexpected chromosomal rearrangements. Although most cultivated forms of peas from the northern temperate zone are believed to possess the standard karyotype as defined in Blixt (1958), some spontaneous translations have been identified in European cultivars (see references in Yarnell 1962). Lamprecht (1939) had observed the spontaneous appearance of new chromosomal rearrangements in his experimental material with an average frequency of ~1/100,000, but in some lines reciprocal translocations occurred with the frequency as high as 1/1,000 (Lamprecht 1949).

In this communication we report the location of the gene *nod-3* as well as the presence of a novel translocation in the mutant line Nod 3 and its parent, the cultivar Rondo.

Materials and Methods

Seeds of the mutant line Nod 3 and its parent cv. Rondo were kindly provided by Dr. Jacobsen (University of Groningen, The Netherlands). Multiple marker lines JI 73 (NGB 1238; obtained from the John Innes Institute, Norwich, U.K.) and Slow (developed at Cornell University, Ithaca, New York) were used as alternative parents for crossing with the Nod 3 line. Both marker lines have normal karyotype and are recommended for mapping studies in pea (Weeden et al. 1993). Nod 3 × JI 73 will be referred as cross A, and Nod 3 × Slow as cross B.

Plants were grown in small conical pots (50 cm^3) containing vermiculite and watered with a low N-nutrient solution. Seedlings were inoculated with *R. leguminosarum*, strain 128C53, grown in a 20°/15°C 16 h/18 h day/night regime in a light room, and scored for nodulation at 3–4 weeks after planting as described earlier (Kneen and LaRue 1988). After nodulation tests, plants were transplanted into potting soil, and scored later for morphological markers, isozyme markers, and pollen fertility.

Isocitrate dehydrogenase phenotypes were determined as described (Weeden

Table 1. Joint segregation analysis for genes *nod-3*, *d*, and *Idh*

Cross	Gene pair	No. plants with designated phenotype[a]						N	χ^2	P[b]	Recomb. fraction[c]
		+/1	+/h	+/2	−/1	−/h	−/2				
A	*nod-3/d*	43	—	28	26	—	0	97	14.4	$<10^{-6}$	<5 ± undefined
A	*nod-3/Idh*	1	32	23	10	6	0	72	37.5	$<10^{-6}$	11 ± 4
B	*nod-3/Idh*	14	51	38	21	14	1	139	32.9	$<10^{-6}$	23 ± 4

[a] Phenotype designations: + = normal nodulation; − = hypernodulation; 1 = mutant line Nod 3; 2 = marker line Slow or Jl 73; h = heterozygous.

[b] Probability (P) based on 9:3:3:1 or 3:6:3:1:2:1 phenotypic ratios for random assortment.

[c] Recombination fraction ± standard error.

[d] No plants expected in categories marked "—".

and Marx 1984) except that electrophoresis was performed on 94 × 76 mm cellulose acetate plates (Helena Laboratories, Beaumont, Texas). The standard staining procedure was modified for applying ingredients in low gelling temperature agarose (FMC BioProducts, Rockland, New York) to the plate surface.

Estimation of pollen fertility was carried out with 4% acetocarmine: glycerin (1:1). Preparation and staining of meiotic chromosomes in acetocarmine were performed according to standard procedure (Lamm 1951).

The computer program LINKAGE-1 was used for segregation analysis (Suiter et al. 1983). In cases involving a translocation point, the data were analyzed according to Lamm (1950). Based on this method, three components of the total χ^2 were calculated, representing the segregation of fertility versus semisterility ($\chi^2_{fert.}$), the segregation of the allelomorphs ($\chi^2_{allel.}$), and the linkage component ($\chi^2_{link.}$).

Results and Discussion

Linkage was initially detected between genes *nod-3* and *D* after scoring F_2 plants from cross A for nodulation and presence of the anthocyanin ring at the base of the stipules (Table 1). However, the mutant and morphological markers were in repulsion phase, and the recombination fraction could not be determined precisely. Both F_2 mapping populations segregated at the locus *Idh*, coding isocitrate dehydrogenase. We obtained a recombination value of 11 ± 4% between *nod-3* and *Idh* in cross A and 23 ± 4% in cross B (Table 1).

Other genes involved in nodulation and nitrogen fixation had been previously mapped to this linkage segment: *sym-2* (Young 1985), *sym-5*, and *sym-19* (Weeden et al. 1990) and locus *Lb*, coding leghemoglobin (Weeden 1990). Postma et al. (1988) reported independent assortment between *nod-3* and *sym-19*. The results of Postma et al. (1988) in combination with our data suggest that genes *nod-3* and *sym-19* must be located on opposite sides of *Idh*. Further detailed genetic analysis is currently in progress for a more precise localization of the genes within the region.

F_1 hybrids between lines Nod 3 and Jl 73 as well as between Nod 3 and Slow were semisterile. Pods on these plants exhibited gaps between seeds and ~35%–40% of the pollen grains were sterile. These observations suggested the presence of a translocation in the line Nod 3, and this translocation was demonstrated cytologically. Configuration of one ring of four and five bivalents was seen at metaphase I in meiosis in pollen mother cells of the F_1 hybrids (not shown). Semisterile plants also were found in samples of Rondo, indicating that this parental line is heterozygous for translocation and consists of plants with different chromosomal structural types.

F_2 plants of both crosses were scored for several morphological markers (*a, wb, k, b, tl, gp, oh*) and for pollen fertility. Results of the segregation analysis (Table 2) revealed that semisterility cosegregated with gene *b* and did not show linkage with any other markers, including *D* and *Idh*. Linkage between breakpoint and gene *b* (pink flowers) of the linkage group III indicates that the corresponding chromosome 5 is involved in the translocation. The translocation does not appear to effect segregation of the gene *nod-3*. Thus, we consider linkage between *nod-3* and *Idh* to represent true synteny and not be produced by the translocation. However, the finding of semisterile plants in Rondo suggests that other symbiotic mutants obtained from this cultivar may carry the same translocation. In the future, results of mapping studies involving such mutants should be interpreted cautiously.

Table 2. F_2 segregation data relating to the location of the breakpoint of the translocation found in Rondo

Cross	Gene pair	xY	xh	xy	XY	Xh	Xy	N	χ^2 fert.	χ^2 allel.	χ^2 link.	Recombin. fraction
A	*T-nod3*	38	—	13	54	—	18	123	2.79	0.05	0.29	Free segreg.
	T-a	36	—	17	54	—	17	124	2.61	0.39	0.87	—
	T-d	22	—	16	45	—	13	96	4.20	1.39	3.56	—
	T-b	11	—	23	53	—	1	88	4.55	0.31	42.56	3.6 ± 2.9
	T-wb	40	—	12	58	—	14	124	3.23	1.07	0.39	Free segreg.
	T-k	38	—	15	52	—	19	124	2.61	0.39	0.11	—
	T-gp	39	—	13	54	—	17	123	2.9	0.02	0.02	—
	T-tl	14	31	7	19	36	17	124	3.23	2.11	1.21	—
B	*T-Idh*	14	26	15	20	37	16	128	3.13	0.17	0.1	—
	T-oh	25	—	11	31	—	17	74	1.95	3.53	0.65	—

x = fertile; X = semisterile; y = recessive; h = heterozygous; Y = dominant phenotype for the morphological marker. For isozyme marker *Idh* capital Y stands for the allele of the line Nod 3.

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Light Down Lethal: A New Autosomal Recessive Down Color Mutation in Japanese Quail

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A new plumage color mutation of Japanese quail (*Coturnix japonica*) was named "light down lethal" based on its light coloration of neonatal plumage and almost total mortality by 3 weeks of age. Prehatch mortality of the light down lethal was also higher (36.8%) than the wild type (8.2%). The majority of the light down lethal chicks that hatched died within 1 week of age. Among the 103 light down lethal chicks that hatched, only two males survived to adulthood and reproduced normally. Their adult mutant plumage was very similar to that of the wild type. The mutant down showed the same striped pattern on the dorsal surface as the wild-type chick, but the color of the stripes was lighter than the wild type. Genetic analysis revealed that the mutant phenotype is controlled by an autosomal recessive gene. The proposed gene symbol is *ldl*.

In November 1992, a chick having lighter down color than the wild type was found among the progeny from a pair of wild-type Japanese quail (*Coturnix japonica*) maintained at the Department of Laboratory Animal Science, College of Agriculture, University of Osaka Prefecture. Although the chick possessed the same striped pattern on the dorsal surface as the wild type, blurry black and dilute tan stripes replaced, respectively, the dense black and tan stripes of the wild type (Figure 1). The ventral surface of the chick was muddy cream, which was also lighter than the mossy color of the wild type. Eye coloration, however, appeared to be the same as that of the wild type. The original pair produced 43 chicks, of which seven had dilute neonatal plumage. These unusual chicks never survived beyond 8 days of age.

Although a number of plumage color mutations of Japanese quail have been reported (Cheng and Kimura 1990), there is no reported mutation that involves both dilution in down color and nearly total mortality at the chick stage. This article describes genetic analysis for the new phenotype.

Materials and Methods

Care of Quail

I gathered eggs daily from pair matings, stored them in a cooler (15°C), and set them in a Murai MIC-14 C incubator (Murai Incubator Co., Sakurada-cho, Atsuta-ku, Nagoya 456, Japan) every 7 days. The temperature and relative humidity of the incubator were maintained at 37.7°C ± 0.2°C and 70%, respectively. At 10 days of incubation, candling was performed, and I scored fertility and embryonic mortality by inspecting the contents of nondeveloping eggs. The majority of chicks hatched at 17 days of incubation, but they were kept in the incubator for the following day. At 18 days of incubation, chicks were transferred into a Zenkeien M-type brooder (Zenkeien-seisakujo Co., Miyakoda-cho, Hamamatsu-shi, Shizuoka-ken 431-21, Japan) with continuous lighting (2 watt); I kept them at 36°C ± 2°C. The temperature of the brooder was lowered to 33°C ± 2°C when chicks were 2 weeks old. At 4 weeks of age, chicks were transferred into a second Zenkeien M-type brooder and kept at 28°C ± 2°C with continuous lighting (2 watt). At 6 weeks of age and avoiding sib matings, birds were paired in wire cages and kept in the condition of 23°C ± 2°C and a 14-h light : 10-h dark photoperiod. I fed chicks up to 4 weeks old a commercial Japanese quail chick ration (Tokai-kigyo Co., Nishi-ohyama, Ohyama-cho, Toyohashi-shi, Aichi-ken 441, Japan) with 24.5% protein. I fed birds after 4 weeks of age a commercial Japanese quail ration for the laying hen (Tokai-kigyo Co.) with 24% protein. Feed and water were supplied for ad libitum consumption.

Genetic Analysis

I made full-sib matings (single female versus single male) using the wild-type progeny from the original pair. These matings identified carriers of the apparent mutation. Females and males of these full-sib carrier matings were then separated for 2 weeks. The proven carriers were then paired with wild-type controls from the UOP-WT line (Ito and Tsudzuki 1994) to produce F_1 progeny (carrier × control matings).

The F_1 birds were paired among themselves avoiding sib matings, and incidence of carrier matings was noted. I examined the segregation ratio of normal and mutant chicks in the progeny of these carrier

Table 1. Incidence of the light down lethal mutant in carrier matings

No. of eggs set	No. of eggs fertile	No. of dead or pipping embryos[a]			No. of chicks hatched[a]		Total[a]		χ^{2c}	
		1–10 d[b]	After 10 d							
			WT	LD	WT	LD	WT	LD	Chicks	Total
812	804	30	48	70	536	120	584	190	15.74	0.08

[a] WT = wild type, LD = light down lethal.
[b] Days of incubation.
[c] Based on simple autosomal recessive inheritance (wild type : light down lethal = 3:1).