Easter[22] and Ascension[23] islands. However, in these examples the extent of contamination appears to be far less widespread than in the Indian Ocean. There may be several reasons for this. First, the Indian Ocean is a relatively compact, youthful ocean, and it originated from a locus at the Kerguelen–Heard plume. Second, the plume is very distinct from MORB in having a strong Dupal character. The component is thus more easily detected in the MORBs than are the Icelandic and Ascension signatures. Third, the plume has interacted with major spreading axes throughout its history, and therefore has had access to the Indian Ocean mantle system (the other main Dupal islands, Gough and Tristan da Cunha in the South Atlantic, have had relatively localized access to the Atlantic system); and finally, the plume appears to have been active for a long period of time, and there is strong evidence (mentioned above) that it reactivated the sub-continental lithosphere above the embryonic Indian Ocean asthenosphere. The plume could therefore have been actively contaminating the asthenosphere before the break-up of Gondwana.

Identification of mantle domains associated with specific mantle plumes can only be achieved by detailed dredging of the present-day ridge system. Klein *et al.*[3] have recently identified a potential boundary between two oceanic mantle domains, which may represent the eastern influence of the Kergulen plume system. There are too few data to identify the western limit of the influence of the KHP; the basalts of the South-West Indian Ridge appear to have a Walvis Ridge component[2], indicating the influence of another Southern Hemisphere Dupal domain. The unknown factor is the extent to which many of the distinctive Dupal characteristics—in plumes or MORBs—result from long-term residence beneath the stable Gondwana supercontinent.

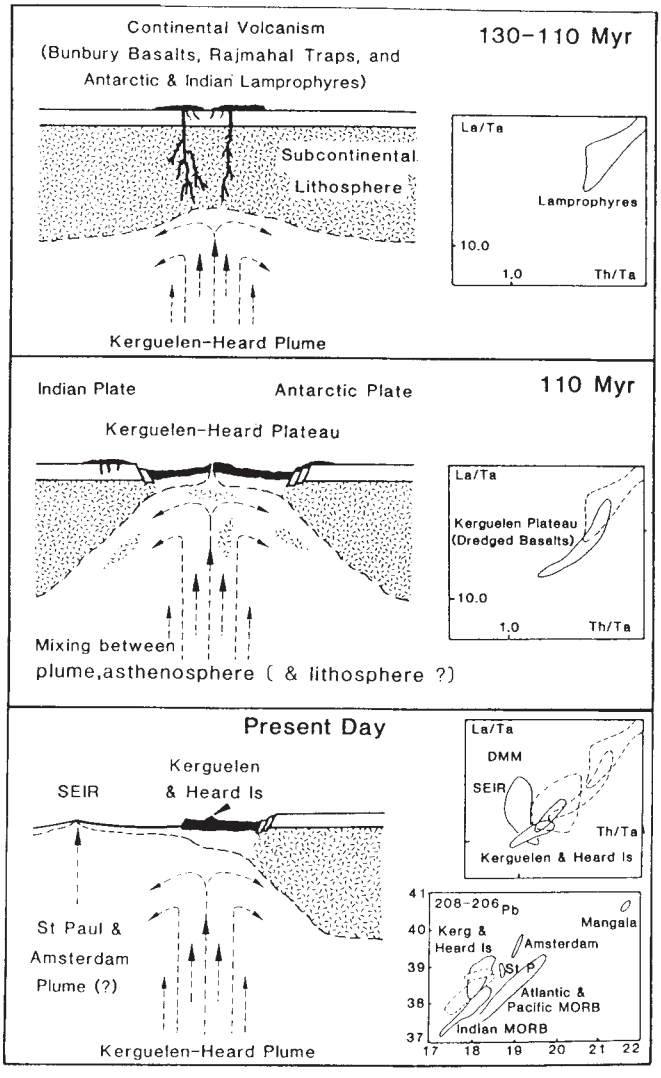

FIG. 4 Schematic diagrams to illustrate the interaction of the Kerguelen–Heard Plume with the sub-Gondwana lithosphere and with the sub-Indian Ocean asthenosphere. See text and Figs. 1–3 for discussion and data sources. DMM: Depleted MORB mantle (see ref. 15).

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Genetic specialists, kin recognition and nepotism in honey-bee colonies

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INSECT societies have long served as useful models in the study of two often intertwined issues in evolutionary biology: the levels at which natural selection operates[1], and the phenomena of competition and cooperation in animal societies[2]. Hamilton[3–5] provided a darwinian explanation for the evolution of cooperation by invoking natural selection for increased inclusive fitness, whereas Trivers and Hare[6] showed how asymmetrical genetic relationships among members of hymenopteran societies result in differential fitness among queens and workers and can lead to conflict over the production of males. Here we provide evidence for competition among workers of the honey bee, *Apis mellifera*, in the production of female reproductives, a consequence of the genetic structure of colonies resulting from polyandry[7], genotypic biases in components of cooperative behaviour associated with division of labour[8–10], and kin recognition[11–13]. We also propose that nepotistic behaviour by honey bees is influenced by both kin- and colony-level selection.

Honey-bee colonies normally consist of about 17 subfamilies[7]. Members of the same subfamily, super sisters[14], share both a

queen mother and a drone father and, in the absence of inbreeding, have a coefficient of genetic relationship of 0.75 (ref. 15). Half-sisters belong to different subfamilies, share only a mother, and have a genetic relationship of 0.25. We tested the ability of workers to discriminate during the rearing of queens in colonies that were derived from instrumentally inseminated queens and composed of three biochemically-distinct subfamilies. Samples of immature (larvae and prepupae) queens and workers, and adult workers located near either immature queens or workers, were collected from experimental colonies and analysed by protein electrophoresis to determine the frequencies of individuals belonging to each of the three subfamilies (see Fig. 1 and Table 1 for details).

Any observed subfamily biases in queen production could be a consequence of intracolonial kin recognition coupled with one or more of four mechanisms: (1) workers preferentially raise the most familiar larvae, that is, those belonging to the subfamily with the highest larval frequency; (2) workers preferentially raise the rarest larvae; (3) the subfamily with the most adult members on queen cells dominates queen rearing and preferentially raises super sisters; and (4) biases occur in the proportional representation of subfamilies engaged in the rearing of queens (relative to larval frequencies) and individuals preferentially rear super sisters. To determine which, if any, of these mechanisms are involved, four series of Monte Carlo simulations were conducted based on the results of our allozyme analyses. Each series of simulations focused on a different 'test'-subfamily that was appropriate to each one of these four possible mechanisms.

Our results demonstrate nepotism during the rearing of queens. Individual workers were located on or off queen cells, at least in part, on the basis of genotypically determined factors. Treatment of immature queens (involving perhaps differential feeding and/or selective removal) was apparently mediated by genetic relatedness with a small but consistent rearing preference shown for more closely related larvae. Subfamilies with a bias

TABLE 1 Nepotism in the rearing of queens by honey bees

		Test-subfamily						Test-subfamily			
Total no. immature workers analysed	Total no. queens analysed	Weighted average of frequency in immature worker and queen samples	Expected no. queens reared	Observed no. queens reared	Deviation	Total no. immature workers analysed	Total no. queens analysed	Weighted average of frequency in immature worker and queen samples	Expected no. queens reared	Observed no. queens reared	Deviation
		Colony 4438						Colony 4450			
49	29	0.46	12	15	+3	40	32	0.07	0	5	+5
50	28	0.37	10	11	+1	40	38	0.35	11	15	+4
39	7	0.33	2	3	+1	40	14	0.00	0	0	0
		Colony 4439						Colony 4453			
40	6	0.35	2	3	+1	40	25	0.45	10	12	+2
40	24	0.38	10	8	−2	40	26	0.24	6	6	0
40	7	0.26	2	0	−2	40	17	0.28	5	3	−2
		Colony 4440						Colony 4456			
50	33	0.30	9	11	+2	49	32	0.19	6	5	−1
41	44	0.39	13	21	+8	51	44	0.40	15	20	+5
40	28	0.31	8	9	+1	40	37	0.41	14	17	+3
		Colony 4442						Colony 4457			
40	31	0.39	11	6	−5	40	36	0.38	10	18	+8
40	40	0.40	14	18	+4	40	40	0.55	19	25	+6
40	50	0.10	2	7	+5	40	53	0.28	15	15	0
		Colony 4445						Colony 4464			
50	35	0.29	8	13	+5	40	29	0.06	1	2	+1
50	41	0.13	2	9	+7	40	36	0.04	1	2	+1
41	25	0.23	6	6	0	34	32	0.64	21	20	−1
										Total deviation = +60	

The table demonstrates nepotism in the rearing of queens by honey bees based on kin recognition and subfamily differences in the likelihood of raising queens (mechanism 4 in text). In this case the 'test'-subfamily (one per trial for each colony, three trials total) was selected because it exhibited a higher probability of being located on queen cells than other subfamilies in the colony. For the analysis, four different sets of 'test'-subfamilies were selected, each set appropriate to one of the four hypothesized mechanisms underlying biases in the rearing of queens. To test the hypothesis of mechanism 4 we determined the relative likelihood of rearing queens, RL, for individuals of each subfamily (s) within a colony: $RL_S = PA_S/PI_S$, where PA_S is the proportion of subfamily S in sample of adults on queen cells, and PI_S is the proportion of subfamily S in sample of immature workers. The subfamily with the highest value of RL for each colony in each trial was designated the 'test'-subfamily for mechanism 4. The expected number of queens for the test-subfamily $E(Q_t)$ was then calculated from the equation $E(Q_t) = (I_t)(N)$, where I_t is the proportion of test-subfamily in sample of immature workers, and N is the total number of queens raised in a given trial. The difference between $E(Q_t)$ and the observed number of queens $O(Q_t)$ was then calculated to determine the deviation. Because conventional analyses of contingency tables were inappropriate[16] (workers selected queens without replacement from among a finite sample of 60 transferred larvae), we used Monte Carlo simulations to estimate the probability of getting a deviation that was as great or greater than our result. For each of the four hypothesized mechanisms, the Monte Carlo simulation randomly drew a number of immature workers equal to the actual sample from a very large (infinite) pool with appropriate test-subfamily frequencies equal to the weighted average of the immature worker and queen samples. It then drew an independent random sample of immature queens from the same pool and calculated the deviation from expected as described above. Deviations were summed for all trials of all colonies. The simulation was iterated 1,000 times and iterations in which the total deviation was as great or greater than that observed were summed. The probability of getting a result as extreme as that observed was calculated as the number of iterations with a total deviation greater than that observed divided by 1,000. The entire simulation process was repeated five times to give an estimate of the standard error of the simulation-generated probability. Observed deviations cannot be a consequence of nonrandom sperm usage by queens because $E(Q_t)$ and $O(Q_t)$ were both based on the same samples of immature females.

in adult representation as queen nurses, relative to the available potential queen larvae, biased the colony's output of queens in their favour.

On average, only half ($\bar{X} = 30 \pm 11.8$ s.d.; 916 queens were raised in total) of the 60 transferred larvae were raised during each trial of each colony, thus providing ample opportunity for workers to make 'choices' with respect to which larvae become queens. Combining data from all trials of all colonies, the subfamilies whose adult members had the highest within-trial individual probability of being sampled on queen cells (mechanism 4; Fig. 1) collectively had 60 more super-sister queens raised than expected on the basis of samples of immature workers ($P = 0.001 \pm 0.0002$ s.e., $n = 5$ Monte Carlo simulations; see Table 1). In contrast, members of the numerically most common subfamily, again based on samples of immature workers (mechanism 1), had 37 fewer queens raised than expected. Individuals belonging to the rare subfamily (mechanism 2) had 13 more than expected ($P = 0.179 \pm 0.0066$), and those belonging

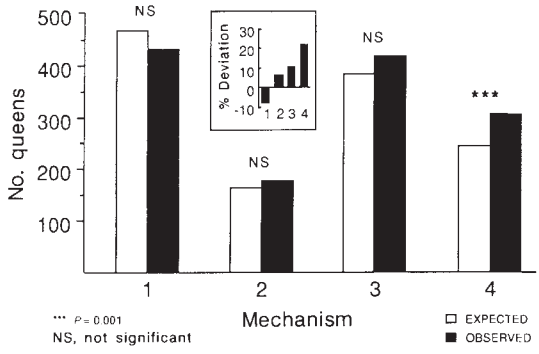

FIG. 1 Expected and observed number of queens reared for each of four mechanisms hypothesized to result (along with kin recognition) in nepotism in the rearing of queens by honey bees. The inset depicts the per cent deviation of the observed from the expected values for each mechanism. For explanation of statistical analyses see legend for Table 1.

METHODS. Ten colonies were established, each composed of three electrophoretically distinguishable subfamilies. The colonies' queens were daughters of three unrelated queen mothers. Drones for matings were progeny of eight additional unrelated queens. Each of the ten queen daughters was instrumentally inseminated[23] with semen from three unrelated drones, each one bearing a different malate dehydrogenase-*1* (Mdh) marker allele, designated 'slow' (S), 'medium' (M) and 'fast' (F)[24]. Semen from each drone trio was pooled, diluted, and mixed before insemination to minimize temporal fluctuations in subfamily frequencies[25,26]. Four of ten inseminated queens were homozygotes at the *Mdh* locus (SS: colonies 4438, 4440, and 4456; MM: colony 4453), producing female progeny with three allozyme phenotypes that correspond to three subfamilies. Five queens (colonies 4439, 4442, 4445, 4450, and 4464) were SF heterozygotes that produced three subfamilies with five allozyme phenotypes: SS, SM, SF, MF, and FF. Larval and adult bees sampled from these colonies were grouped into three subfamilies as follows. SM and MF bees were assigned to the M subfamily because the M allele could only be paternally inherited. SF bees were assigned to the S and F subfamilies in proportion to the ratio of SS and FF phenotypes in each sample, because the maternal S and F alleles are expected to segregate in a 1:1 ratio. Subfamily membership was determined similarly for individuals from colony 4457, derived from a SM queen. The experiment took place from April to May, 1987, under conditions of seasonal production of queens associated with normal colony reproduction through fission[27]. At the start, nine of ten colonies (all except 4445) were raising queens naturally. Colonies occupied hives consisting of two or three chambers; the lower two were filled with combs of brood and honey, and the third with honey and empty combs. Each queen was confined to a bottom chamber of the hive with a queen excluder. For each colony, 60 newly hatched female larvae were transferred from a frame of worker comb containing up to 3,700 individual larvae into dry, artificial queen cells arranged 20 cells to a bar, three bars to a frame (female larvae located in special queen cells are fed differentially and develop into queens rather than workers[28]). The two frames were placed together in the centre of the upper brood chamber in each hive, with the queen cells closest to the side from which larvae were transferred. Three days later we removed the two frames, with minimal disturbance, and collected ~50 workers each from the surface of the queen cells and from the area of worker comb that originally contained the transferred larvae. These were assumed to be queen and worker 'nurses', respectively. Immatures (larvae and prepupae) were sampled six days after transfers when all investment in new queens was complete and the queen cells were capped by the workers[28]. In addition to collecting all queen cells, we gathered samples of about 50 immature workers that were the same age and located in the same area of the comb as were the queen larvae before they were transferred. All samples were stored at −70 °C until analysed by electrophoresis. Each colony was tested three times at 7–8-day intervals for a total of 30 trials. All queens, 34–51 immature workers, and 34–43 adult workers per sample were analysed.

to the subfamily with the highest frequency of adult workers on queen cells, regardless of numerical representation in the colony (mechanism 3), had an excess of 34 raised ($P = 0.064 \pm 0.0034$). In 13 of the 18 trials that showed a positive bias in the rearing of queens, assuming mechanism 3, the numerically dominant adult subfamily was also the one whose members had the greatest bias in likelihood of being sampled as queen nurses and, therefore, were also classified as the test subfamily based on mechanism 4. In these cases, mechanisms 3 and 4 were confounding.

Genetic 'specialization' for rearing queens is suggested by the following results. In four of ten colonies, the subfamily distribution of adult workers sampled on queen cells differed significantly from the distribution of adult workers sampled on the adjacent comb containing worker larvae in at least one of three trials, even though the sampled frames were only ~2–4 cm apart ($P < 0.05$, colony 4440; $P < 0.01$, 4450; $P < 0.01$, 4456; $P < 0.05$ and $P < 0.01$, 4464; G tests of heterogeneity[16]). In two colonies subfamily differences were significant for the three trials combined ($P < 0.01$, 4453 and $P < 0.05$, 4456). The number of trials that showed a significant deviation ($P < 0.05$) was greater than that expected based on chance alone ($P < 0.02$; binomial test, assuming 30 independent trials). These differences may be due either to paternally inherited behavioural traits that generate a bias in the representation of adult subfamily members on queen cells relative to their representation in the pool of potential queen zygotes, or, alternatively, to nonrandom sperm use by queens coupled with age differences between queen 'nurses' and worker 'nurses'. Results based on analyses of patterns of sperm use for each queen do not support the alternative explanation. The subfamily distribution in worker larvae samples fluctuated significantly among trials in only two of ten colonies ($P < 0.01$, 4445 and 4450), suggesting that the sperm of the three drones used for each insemination were well mixed in eight colonies.

Subfamily biases for the rearing of queens were small and consistent with previous studies. The ratio of the proportion of test-subfamily (assuming mechanism 4) adults sampled on queen cells to its proportion in the sample of worker larvae (see Table 1 legend) was 2.3 ± 1.90 s.d. The mean ratio of the observed to the expected number of test-subfamily (mechanism 4) queens raised, based on the worker larval samples, was 1.5 ± 1.10 s.d. Page and Erickson[17] reported a ratio of 1.6 in the number of queens reared when workers were given a choice of super-sister and less related ($G = 0.25$ or 0.31) non-nestmate larvae. Visscher[18] found a bias of 1.5 in favour of a mixture of super- and half-sister nestmate eggs or larvae over unrelated non-nestmates. Noonan[19] demonstrated a bias of 1.2 in the visitation frequency of adult workers to queen cells containing super- versus half-sister larvae in colonies composed of two visually distinguishable subfamilies.

In agreement with previous studies[8–10], our results suggest that the genotypes of workers affect colony organization by influencing the probability of performing behaviour associated with the division of labour. Worker genotype also results in differential social interactions, some of which may lead to intranidal conflict. Simply determining genetic relationships among colony members is not sufficient for testing kin-selection theory; the genetic relationships among interacting individuals must be considered (compare with ref. 20). Our results also demonstrate that kin selection is an important process in the evolution of social interactions involved in reproduction.

Larval kin recognition should increase the adaptive value of genotypes that result in a higher probability of the rearing of queens, driving them rapidly to fixation in populations. Perhaps this is a social analogue of meiotic drive[21] that distorts the proportional representation of queen genotypes (M.D. Breed, personal communication). Genetic variability and the rather modest levels of nepotism observed may be functionally linked and represent a compromise between levels of selection[22]. Kin selection may favour alleles that result in intracolonial competition: high propensity to rear queens, better recognition, and

more discrimination. However, variation in the rearing of queens coupled with larval discrimination may reduce the reproductive output of colonies, a consequence of decreased rearing of queens due to adult-adult or adult-larva conflict, or a decrease in the ergonomic efficiency of the colony. If so, then colony-level selection may favour cooperation: a lower propensity for rearing of queens, and less discrimination. With additional genetic analyses of insect societies, the functional significance of the observed genetic variability, and the mechanisms underlying the intricate interplay of competition and cooperation may be further elucidated. □

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Root lectin as a determinant of host–plant specificity in the *Rhizobium*–legume symbiosis

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THE induction of nitrogen-fixing nodules in legume roots by soil bacteria from the genera *Rhizobium* and *Bradyrhizobium* is host–plant-specific. This specificity is expressed at an early stage of the infection process and results from multiple interactions between bacterial and plant products. Among these, it has been suggested that root lectin recognized by bacterial receptor molecules is an important determinant of host specificity[1,2]. Lectins are carbohydrate-binding proteins and it is known that legumes belonging to different cross-inoculation groups produce lectins with different sugar-binding specificities[3]. We have tested this suggestion by introducing the pea lectin gene into white clover roots using *Agrobacterium rhizogenes* as a vector. The 'hairy' clover roots that result can be nodulated by a *Rhizobium* strain usually specific for plants from the pea cross-inoculation group.

Rhizobium leguminosarum bv. *viciae* nodulates the cross-inoculation group of pea, vetch and lentil, whereas clover is nodulated by *R. leguminosarum* bv. *trifolii*. The involvement of root lectin in determining this host-plant specificity was tested by introducing the pea lectin gene (*psl*) into the roots of white clover and by studying the infection of transgenic clover roots by *R. leguminosarum* bv. *viciae*. Transgenic roots rather than transgenic plants were studied because of the problems of regenerating the latter.

We cloned *psl* into a binary vector[4] using *A. rhizogenes* LBA1334 (*Ar*1334) (ref. 5) to obtain transgenic clover roots. The *psl* gene encodes a glucose/mannose binding protein[6,7] and is expressed at low and developmentally controlled levels in pea roots during the plant's life cycle[8]. Pea lectin is localized at the surface of growing pea root hairs, which are the target cells for rhizobial infection[9]. Lectin-enhanced accumulation of infective rhizobia at pea root-hair tips correlates with increased infectivity of the rhizobial cells[10].

Complete genomic *psl* (*psl* lecA provided by J. A. Gatehouse[7]) was cloned in the binary vector pBin19 (ref. 11). In addition, the full length *psl* complementary DNA[12] (provided by M. Stubbs) was cloned in the binary expression vector pAGS 35S HB (ref. 13) (provided by C. M. P. van Dun) between the plant viral 35S promoter and the nopaline synthase 3′ end. These binary vectors are referred to as pBin19 *psl* and pAGS c*psl*, respectively. (For details of the cloning steps see Fig. 1.)

The efficiencies of the binary vector systems were tested by determining the activity of a reporter gene product. Both vectors carry a neomycin phosphotransferase gene (NPTII, see Fig. 1) which can be expressed in transformed plant tissue. Hairy roots

TABLE 1 Nodulation of white clover hairy roots by *Rhizobium leguminosarum*

Agrobacterium strain and binary vector	No. nodulated plants			
	Inoculation with bv. *trifolii*			
	*10 d	23 d	30 d	40 d
LBA1334	13/20	18/20	20/20	20/20†
LBA1334 pBin19	7/15	14/15	15/15	15/15†
LBA1334 pBin19 *psl*	15/20	19/20	20/20	20/20†
LBA1334 pAGS c*psl*	15/19	19/19	19/19	19/19†
	Inoculation with bv. *viciae*			
LBA1334	0/19	0/19	0/19	1/19‡
LBA1334 pBin19	0/15	0/15	0/15	0/15
LBA1334 pBin19 *psl*	0/20	5/20	8/20	14/20§
LBA1334 pAGS c*psl*	0/19	2/19	6/19	11/19§

Trifolium repens L. (white clover) seedlings were grown with their roots on filter paper in Petri dishes containing Jensen-1% agar[14] supplemented with 0.5 mM $Ca(NO_3)_2$. Transformation was achieved by superficially wounding the stems of 6–8-day-old seedlings 2 mm under the first pair of leaves with the tips of an electron microscope grid forceps (number 7, Dumont, Switzerland) carrying *A. rhizogenes*. Bacteria were grown on LC (ref. 24) plates containing full antibiotic concentrations (see Fig. 1 legend) for *Ar*1334, *Ar*1334 carrying pBin19 or *Ar*1334 harbouring pAGS c*psl* or pBin19 *psl*. Following transformation, the main root was excised 2–3 mm under the wound and the first hairy roots emerged from the wound sites 4–5 days later in all plants. Seedlings were transferred to fresh nutrient plates 5–7 days after wounding and nodulation assays were performed as described previously[14]. For re-isolation of rhizobia, red nodules were surface-disinfected in 5% H_2O_2 for 10 min, rinsed in sterilized water and crushed in 250 μl B-medium[25] and plated on B-medium[25]. Re-isolates from *Rl*248 RifR-induced nodules were rifampicin-resistant, in contrast to re-isolates from *Rt*843-induced nodules. Colonies from re-isolated rhizobia were blotted to nitrocellulose sheets and allowed to react with monoclonal antibody (mAb) 3, specific for the O-antigen of *Rl*248 (ref. 21). Only re-isolates from *Rl*248 RifR-induced nodules were recognized by mAb 3. Rhizobial cell envelopes were analysed by SDS-polyacrylamide gel electrophoresis as previously described[26]. A protein of relative molecular mass 45,000 and characteristic of *Rl*248 RifR (control) was present in bacteria re-isolated from *Rl*248 RifR-induced nodules and absent from re-isolates from *Rt*843-induced nodules. Nodulation of *Vicia sativa* L. by *Rl*248 RifR-re-isolates and their failure to nodulate white clover roots confirmed that *R. leguminosarum* bv. *viciae* 248 RifR nodulates hairy roots of white clover co-transformed with pBin19 *psl* or pAGS c*psl*. White clover seeds of cultivar 'Dutch Clover' (6990.6300) were purchased from Kieft, Blokker, The Netherlands.

* Days after inoculation with rhizobia.
† Red nodules.
‡ Pseudonodules.
§ Red and white nodules and pseudonodules.