Table 1 The observed and expected numbers of herbivores in a grassland insect community

Fortnightly interval	No. of species	Observed no. of herbivores	Expected no. of herbivores	Expected variance in herbivore no.
1	20	12	12.4	4.4
2	32	22	19.9	6.6
3	64	40	39.7	10.8
4	92	62	57.1	12.6
5	116	77	72.0	12.7
6	137	88	85.1	11.7
7	147	98	91.3	10.8
8	150	95	93.2	10.5
9	133	79	82.6	12.0
10	144	91	89.4	11.1
11	127	78	78.9	12.3
12	98	64	60.9	12.8
13	52	29	32.3	9.5
14	22	18	13.7	4.8

The data from the 14 sample intervals of Evans and Murdoch specify all quantities of equations (2) and (3). The number of species is the quantity r. Other quantities are given in the text. The fit of observed values to theoretical expectations is tested by a $\chi^2 = 3.45$, d.f. = 13, $P > 0.995$.

The data from the 14 fortnightly intervals given by Evans and Murdoch are shown in Table 1. The total number of herbivorous and entomophagous species in this grassland community is given as 131 and 80, respectively. This represents values of $n = 211$ and $n_1 = 131$. All values of equations (2) and (3) are known and thus the expected number of herbivores and the expected variance can be calculated.

Figure 1 shows the observed number of herbivores and the expected number of herbivores. Points fall on the line if the expected number of herbivores equals the observed number. The bars through the points are 2 s.d. from the expected values. All but one of the points (fortnightly interval 7) lie within 2 s.d. of the expected value.

There seems to be no systematic tendency in the samples to lie either above or below the line. Four samples are found to have more herbivores than expected, 10 have fewer than expected. The two-tailed binomial probability for this, or a more deviant event, is $P = 0.284$.

There does not seem to be any trend in deviation from the expected as one considers various numbers of species. Two of the four values greater than expectations are above the median of total species, two of the values are below the median. When there are few species, at either the beginning or end of the season, there does not seem to be any less trophic structure. Nor does there seem to be more trophic structure during the period of greatest species diversity.

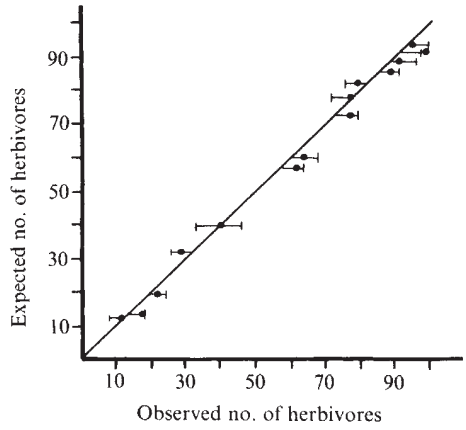

Fig. 1 The expected number of herbivores is plotted against the observed number of herbivores. The bars represent 2 s.d. from the expected number of herbivores.

In conclusion, there does not seem to be any evidence that the ratio of herbivores to predators in this grassland insect community is maintained at a constant level by any other force than a statistical one. Analysis at another, deeper, level, well beyond the scope of this report, might ask why the ratio of herbivorous insects to their predators is 1.64. Given that it is, however, no further evidence for trophic structuring exists.

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Co-transfer of determinants for hydrogenase activity and nodulation ability in *Rhizobium leguminosarum*

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In all organisms that fix atmospheric nitrogen a by-product of the nitrogenase reaction is hydrogen gas[1] which may dissipate up to one-third of the energy flux through nitrogenase[2]. Some nitrogen-fixing bacteria, including certain strains of the root nodule bacterium *Rhizobium*, possess an active hydrogen uptake (Hup) system permitting hydrogen to be re-cycled[3,4]. For this reason Hup$^+$ *Rhizobium* strains are thought to be more energy-efficient symbionts than their Hup$^-$ counterparts[5–8]. We report here that determinants for hydrogenase activity (*hup*) in a particular strain of *R. leguminosarum* (128C53) are genetically linked to determinants for nodulation ability (*nod*), and are probably carried on a plasmid, pRL6JI, of molecular weight (MW)~19×10^7. Although pRL6JI was not self-transmissible, the determinants for nodulation ability and hydrogenase activity (*hup*) could be transferred to other strains of *R. leguminosarum* after recombination with a derivative of a transmissible *R. leguminosarum* plasmid.

Nodulation ability is plasmid-determined in *R. leguminosarum*[9–12] and several determinants for nodule formation and function may be carried on a single plasmid[11–15]. We therefore attempted to transfer determinants for nodulation ability from strain 128C53, a Hup$^+$ field isolate of *R. leguminosarum*[8,16], into a non-nodulating (Nod$^-$) mutant of the Hup$^-$ field-isolate *R. leguminosarum* strain 300 in the hope that determinants for Nod$^+$ and Hup$^+$ were genetically linked and might consequently be co-transferred. The recipient strain was 16015, a derivative of strain 300, which contains *str-37* and *spc-54* markers and a plasmid deletion in a region determining nodule formation (Nod$^+$) and nodule function (Fix$^+$)[11–13].

Strain 128C53 contains two large plasmids (MW~19 and 23×10^7) but no evidence for self-transmissible plasmids was obtained using methods which had identified such plasmids in other *Rhizobium* strains[9,14,17]. Therefore two self-transmissible plasmids, which did not themselves suppress the Nod$^-$ phenotype of strain 16015 but which were known to mobilize nodulation plasmids from other strains[13], were introduced into strain 128C53. The transmissible plasmids used were pVW3JI and pVW5JI, kanamycin-resistant derivatives of pRL3JI and pRL4JI respectively[13].

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Table 1 Co-transfer of Nod$^+$ and Hup$^+$ determinants from derivatives of strain 128C53 to strain 16015 following recombination with transmissible plasmids determining kanamycin resistance

Donor strain	Plasmid from which *kan* marker was derived	Recipient strain	Frequency of transfer of *kan* per recipient	No. of Kan^r transconjugant clones tested on plants			
				Total	Nod$^+$	Fix$^+$	Hup$^+$
a							
2515	pVW3JI	128C53	2×10^{-4}	5	5	5	5
2517	pVW5JI	128C53	1×10^{-4}	5	5	5	5
b							
3856	pVW3JI	16015	5×10^{-7}	15	11	11	11
3957	pVW5JI	16015	5×10^{-7}	15	10	10	10
c							
2515	pVW3JI	16015	3×10^{-2}	10	0	–	–
2517	pVW5JI	16015	5×10^{-2}	10	0	–	–

a, Introduction of transmissible plasmids into 128C53 derivatives; *b*, transfer of plasmid-linked determinants from 128C53 derivatives to the non-nodulating strain 16015; *c*, control crosses: direct transfer of plasmids from 300 derivatives to strain 16015. Two derivatives of strain 300, each carrying a different transmissible plasmid conferring kanamycin resistance, were mated with strain 3854, a spontaneous *rif* mutant of strain 128C53 (itself a Hup$^+$ field isolate of *R. leguminosarum*). The genotypes of the donor strains were as follows:– 2515:300 *phe-1 ade-27 rif-45* pVW3JI; 2517:300 *phe-1 ade-27 rif-45* pVW5JI. Kanamycin-resistant derivatives of 3854 (128C53 *rif-397*) were obtained by introducing pVW3JI or pVW5JI into this strain and these derivatives were termed 3856, 3957 respectively (*a*). These two strains, together with the corresponding strain 300 donors were used in crosses to the Nod$^-$ strain 16015 (*b* and *c*). Kan^r derivatives of 16015 were repurified and scored for nodulation ability on duplicate peas, var. Wisconsin Perfection. Roots of nodulated plants were tested for acetylene reduction (Fix$^+$) and for the incorporation of tritiated hydrogen gas (Hup$^+$) into the aqueous phase as described in Table 2.

When the 128C53 derivatives into which pVW3JI or pVW5JI had been introduced were used as donors to strain 16015, transfer of *kan* occurred at low frequency (10^{-6} to 10^{-7} per recipient). However, more than half of the 16015 transconjugant clones were now able to nodulate peas (Table 1). In all cases the nodules formed had hydrogenase activity comparable to that for strain 128C53 and the rate of hydrogen evolution was correspondingly diminished compared with a Hup$^-$ strain (Table 2).

Because strain 300 itself is Hup$^-$ and neither Nod$^+$ nor Hup$^+$ phenotypes are conferred by pVW3JI or pVW5JI (data not shown), both Nod$^+$ and Hup$^+$ determinants must have been derived from strain 128C53 and must therefore be genetically linked in that strain. This was confirmed in further crosses between the 16015 Kan^r Nod$^+$ Hup$^+$ transconjugants and strain 6015 (which carries the same *nod-6007* deletion as strain 16015[9]: in these crosses *kan* was transferred at high frequency (5×10^{-3} per recipient) and most (22/25) of the Kan^r 6015 transconjugants were also Nod$^+$. In all cases Nod$^+$ transconjugants were Hup$^+$. The very high-frequency co-transfer of *nod* and *hup* with *kan* suggests that there has been genetic recombination between these markers, and the high-frequency transfer of *kan* suggests the involvement of a plasmid, at least at this stage.

The transfer frequency of *kan* from the 128C53 derivatives to strain 16015 was inexplicably low but was at least 10-fold higher than for transfer of chromosomal alleles (data not shown). When similar crosses were performed using the P1 group R plasmid pJB3JI (which confers tetracycline resistance[14]), the Tet^r determinant was transferred into and out of strain 128C53 at normal frequencies (10^{-2}–10^{-3}), but no co-transfer of Nod$^+$ to strain 16015 was observed (data not shown).

Because the Nod$^+$ determinants of strain 128C53 were probably plasmid-borne, the plasmids from many of the strains described here were examined after electrophoresis on agarose gels (Fig. 1). In strain 2515, a 300 derivative, the plasmid pVW3JI was visible as the second-fastest migrating band, MW 13×10^7 (Fig. 1 track *a*, and see ref. 12). However, in all three Kan^r derivatives of 128C53 examined after transfer of pVW3JI into this strain, no band corresponding to pVW3JI was visible (Fig. 1 track *c*): furthermore, the smaller of the two plasmids (MW 19×10^7) visible in strain 128C53 (Fig. 1 track *b*) had also disappeared. An identical plasmid pattern was observed in Kan^r derivatives of 128C53 obtained after the transfer of pVW5JI (MW 16×10^7) into this strain. However, when the P1-group plasmid pJB3JI was transferred into strain 128C53 (Fig. 1, track *e*) the two resident plasmids of strain 128C53 remained visible and in addition there was a band corresponding in mobility to that of pJB3JI.

The simplest interpretation that is consistent with both the genetical and physical observations described is that the smaller plasmid of strain 128C53 is the likely carrier of the linked Nod$^+$ and Hup$^+$ determinants. We term this plasmid pRL6JI, and we propose that pRL6JI recombined (or co-integrated) with the introduced plasmid to form a transmissible plasmid carrying determinants for Nod$^+$ and Hup$^+$ from pRL6JI, Kan^r from

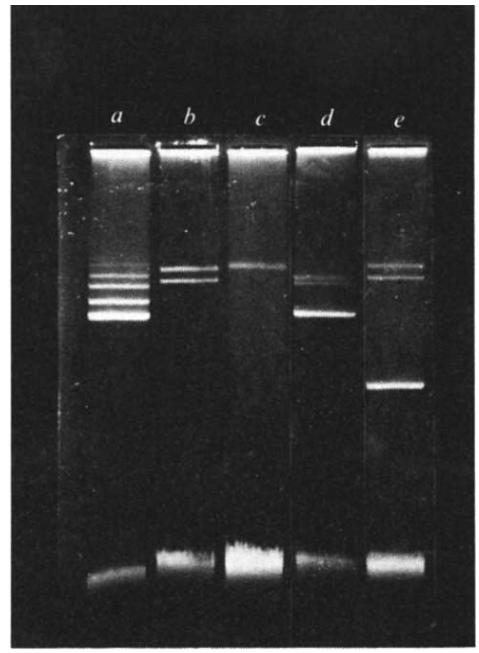

Fig. 1 Physical interaction between pVW3JI and a plasmid from strain 128C53. See Tables 1 and 2 for a description of the relevant strains. Lysates were prepared and analysed by electrophoresis on agarose gels following the method of Hirsch *et al.*[12]. In each track the fastest migrating band represents sheared (chromosomal) DNA. *a*, Strain 2515. The plasmid band corresponding to pVW3JI is the second-fastest migrating plasmid band. The two high molecular weight plasmids of strain 300 are just visible on this gel (see ref. 12). *b*, Strain 3854 = 128C53 *rif-397*. *c*, Strain 3856 = 3854 carrying *kan* determinant from pVW3JI. *d*, Strain 3892 = kan^r Nod$^+$ Fix$^+$ Hup$^+$ transconjugant from the cross 3856 × 16015. *e*, Strain 3955 = 3854 pJB3JI. Two tracks have been removed from this gel corresponding to lysates from 3957 (pVW5JI derivative, identical to track *c*) and 16015 (identical to track *d*).

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pVW3JI (or pVW5JI) and Fix$^+$ determinants from either or both moieties. The failure to detect the recombinant plasmid as a band on agarose gels might be due to the fact that its very large size (MW $> 30 \times 10^7$) would make it too susceptible to breakage to be recovered intact in our plasmid preparations. Inter-plasmid recombination might have occurred if pRL6JI shared homologous sequences with pVW3JI and pVW5JI.

There are at least two explanations for the fact that the introduced plasmid was never observed in Kan^r derivatives of strain 128C53. Either the introduced plasmids are incompatible with pRL6JI which itself might carry genes essential for the growth of this strain or, alternatively, pVW3JI or pVW5JI might be incapable of autonomous replication within this host. Both possibilities are consistent with the relatively low initial transfer frequency (10^{-4}) of the transmissible plasmids into strain 128C53 and with the observed disappearance of the band corresponding to pRL6JI from Kan^r transconjugants.

Table 2. Quantitative measurements of pea root nodule activity following inoculation with Hup$^+$ and Hup$^-$ strains of *R. leguminosarum*

Inoculant	Acetylene reduction (μmole C_2H_4 per plant per h)	Hydrogen evolution μmole H_2 per plant per h	Tritum incorporation (μmole H-T per g-nodule fresh wt per h)
300	5.11 (±0.32)	3.16 (±0.23)	0.002 (±0.001)
300 pRL3JI	8.81 (±0.77)	4.57 (±0.69)	0.001 (±0.001)
3740	8.46 (±0.24)	5.01 (±0.30)	0.001 (±0.001)
128C53	7.97 (±0.99)	0.57 (±0.20)	0.738 (±0.063)
3892	6.84 (±0.48)	0.32 (±0.04)	1.013 (±0.112)
3894	9.43 (±1.09)	0.37 (±0.04)	0.844 (±0.074)

'Alaska' peas grown in modified Leonard-jar assemblies were assayed 25 days after inoculation as previously described[8]. Tritium incorporation by nodulated roots was measured in 25-ml incubation vessels containing tritiated hydrogen gas (2.4%, v/v, 2.4 mCi mmol^{-1}) in the presence of acetylene (10%, v/v) to inhibit hydrogen production by nitrogenase[8]. Incubation was for 30 min and means (± s.e.) were computed for six replicates. Bacteria recovered from surface-sterilized nodules were tested for the appropriate drug-resistance markers and plasmid patterns. Strains 3740, 3892 and 3894 were all Nod$^+$ Kan^r derivatives of strain 16015. In strain 3740, which was Hup$^-$, *kan* was derived from pVW5JI and *nod* from strain 300 (see ref. 13 for the construction of this strain). In strains 3892 and 3894, *kan* was derived from pVW3JI and pVW5JI respectively, *nod* and *hup* determinants were derived from 128C53 (see Table 1).

The plasmids of the 300 Nod$^-$ strain 16015 have already been described[12,14]. In the Kan^r derivatives that were Nod$^+$ Hup$^+$, no new plasmid bands could be seen (Fig. 1 track *d*). This implies either that any co-transferred plasmid co-migrated on gels with a resident plasmid from strain 16015 or that the co-transferred plasmid was too large to be visualized on gels (as was suggested for the donor strain itself). However, Kan^r 16015 derivatives that were Nod$^-$ (Table 1*b*) contained a new plasmid band present in neither strain 16015 nor in the donor strain, but corresponding in size to the original plasmid band of pVW3JI as seen in lysates of normal strain 300 derivatives (Fig. 1 track *a*). In these cases it seems that the original pVW3JI plasmid may have been regenerated by a reversal of the original recombination event that caused its disappearance from derivatives of 128C53.

We have demonstrated that two apparently unrelated symbiotic phenotypes, nodulation ability for peas and the presence of hydrogenase activity, are genetically linked in strain 128C53. It is possible that a large proportion of the genes concerned specifically with the nitrogen-fixing symbiosis, the so-called symbiotic genes, will be found to be clustered and located on one or a few plasmids. Such an arrangement would certainly make it easier to develop genetically improved strains of *Rhizobium*.

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Note added in proof: A. H. Christensen and K. R. Schubert (personal communication) have informed us that their culture of 128C53 (obtained from Dr J. C. Burton) has a different plasmid profile from that described here for our culture of this strain. It is not clear whether our Hup$^+$ strain has undergone plasmid rearrangements during its history or whether two different strains exist bearing the designation 128C53.

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Nonrandom segregation of nucleolar organizing chromosomes at mitosis?

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The random assortment of non-homologous chromosomes at meiosis is one of the fundamental tenets of genetics, to which few exceptions have been documented[1]. The segregation of mitotic chromatids is believed to be similarly random. We report here that we seem to have discovered a new exception to this rule, in that nucleolar organizing chromosomes remain associated with one another, held in the same lateral orientation, for several mitotic cycles.

Five pairs of human chromosomes (nos 13, 14, 15, 21 and 22) carry rDNA sequences in specific chromosome segments, known as nucleolar organizing regions (NORs). The chromosomes are all acrocentrics, and the NORs are on their short arms. More than one chromosome may participate in the formation of a single nucleolus, DNA from their NORs uncoiling and extending deep into the substance of the nucleolus[2]. During mitosis, the nucleoli disappear, but the chromosomes may be seen at metaphase grouped together with their short arms in close proximity. These configurations are called 'satellite associations' (ref. 3). A very close association of nucleoprotein complexes between associated chromosomes has been demonstrated autoradiographically[4].

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