triplet capable of admixing with ground state, as observed for $[Fe^{III}(C_5Me_5)_2]^{\cdot+}[TCNE]^{\cdot-}$, should suffice.
30. Other hexasubstituted benzene dications may also have singlet ground states, and their structures and magnetic properties should be investigated.
31. Elimination of "pseudo-sixfold" symmetry may stabilize the triplet ground state.
32. J. E. Wertz and J. R. Bolton, *Electron Spin Resonance* (Chapman & Hall, New York, 1986), p. 244 and section 10-11.
33. We are indebted to P. J. Krusic and M. D. Ward for providing EPR and electrochemical data, respectively, and for stimulating discussions with them and E. Wasserman. We appreciate the synthetic assistance of C. Vazquez and D. Wipf, as well as the x-ray diffraction assistance of W. Marshall and Faraday susceptibility data taken by R. S. McLean. We also thank K. Mislow for kindly providing a preprint prior to publication.

4 March 1988; accepted 1 April 1988

Virus-Like Particles and a Spider Mite Intimately Associated with a New Disease of Barley

NANCY L. ROBERTSON AND THOMAS W. CARROLL

The malting barley–producing regions in Montana and Canada are threatened with a new virus-like barley disease that appears to be etiologically novel. Ultrathin sections of diseased tissue contained enveloped, filamentous virus-like particles that measured 64 nanometers by 126 to 4000 nanometers. These lengths are unique for plant viruses. Unexpectedly, the spider mite, *Petrobia latens*, which has never been reported to be a vector of a pathogen, was found to transmit the causal agent from diseased plants to healthy barley, while noninfective mites failed to do so unless they were allowed prior access to diseased tissue.

IN 1982, A DISEASE OF BARLEY (*Hordeum vulgare* L.) was discovered in one barley field in the malting barley–producing area of north central Montana. In 1983 and 1984, the disease was also present in other barley fields as distant as 24 km. In 1985, and again in 1986 and 1987, the disease reached epidemic levels in that area and was also identified in five other contiguous counties. Malting barley producers reported that the disease was causing yield losses and preventing them from meeting malting standards.

When ultrathin sections and crude extracts of diseased leaves (*1*) were examined (Zeiss EM 10 CA electron microscope), long filaments about 64 nm in diameter (*2*) were found that met one size criterion for virus particles (*3, 4*). Many of the particles had extraordinary lengths, with some over 4000 nm in ultrathin sections. Such lengths for intact virus particles have not previously been reported for plant viruses but have been documented for viruses in animals (*5*) and insects (*6*). Inasmuch as these virus-like particles (VLPs) appeared only in diseased tissue and morphologically resembled some virus particles, we hypothesized that they were the causal agent of the disease and conducted tests to determine their possible modes of transmission.

The barley seed we collected in 1983 from diseased field plants were sown in the greenhouse and produced no diseased plants (*7*). Healthy barley seedlings did not become diseased in the greenhouse after mechanical inoculation of triturated diseased leaves (*7*). In another greenhouse test, healthy barley seedlings grown in field soil from a diseased site failed to develop disease symptoms (*7*). From 1983 through 1985, we conducted extensive transmission tests with aphid, leafhopper, and thrips species (many of which are known to transmit plant viruses) (*8*) collected from fields of diseased barley. Again, there was no transmission when these species were separately allowed to feed on healthy indicator test seedlings in the greenhouse (*7*).

However, by 1986, infestations of the brown wheat mite, *Petrobia latens* Muller (Acari: Tetranychidae), were observed to occur on seedlings in fields of barley with subsequent development of the disease. Although brown wheat mite infestations appear sporadically in cereal crops throughout the world (*9*), the mite has never been associated with the transmission of a plant pathogen. To test the hypothesis that *P. latens* was a vector for the causal agent of this disease, we collected immature and adult stages of the mites (*10, 11*) from fields of diseased Klages barley in June 1986 by tapping infested leaves, which caused the mites to fall onto a sheet of white paper (*12*). We then allowed the mite population to feed on caged healthy barley seedlings for 2 months in the greenhouse (*13*). Plants were observed for symptoms, and ultrathin sections of leaf samples were examined to confirm the presence or absence of the VLPs.

In June 1987, we collected mites from the same site of diseased Klages barley and obtained another set of mites from a contiguous field of Lew spring wheat, which had only a trace of the disease. These two populations of mites and their progeny were maintained separately in two cages on barley seedlings. Four consecutive sets of seedlings were exposed to those mites in the cages every 3 weeks. Each set contained 16 Dicktoo barley seedlings, which were initially exposed to the mites at the one-leaf stage (four plants per 15-cm-diameter pot). Approximately 400 mites were placed in each of the cages in the beginning of the experiments. At the end of each cycle, mites were aspirated or gently tapped from the infested seedlings and placed onto the new set of seedlings (*14*). We then fumigated these plants for 2 hours with Vapona (dichlorvos) pesticide before transplanting each plant into pots 20 cm in diameter; the plants were grown and observed in the greenhouse for at least eight more weeks under a 16-hour-per-day photoperiod at temperatures between 20° and 27°C. All plants were assayed for the presence or absence of the VLPs by the leaf extract procedure.

At the end of the fourth cycle, we removed 44 mites from the population that originated from the wheat site and divided them into two groups. In parallel transmission tests, one group of the mites was placed on detached diseased leaves and the other

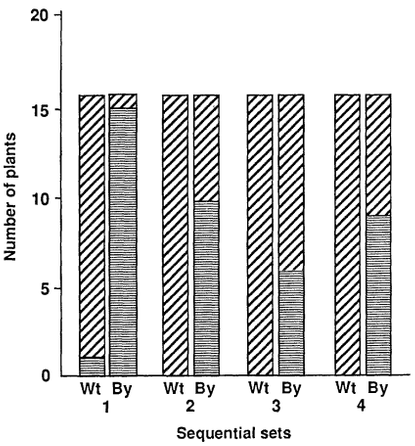

Fig. 1. Effects of exposing healthy barley seedlings to mites that originated from fields of wheat (Wt) or barley (By). Mites derived from healthy-appearing wheat produced only one symptomatic plant in the first set of plants, whereas mites derived from a field of diseased barley were associated with symptomatic plants in every set of plants exposed to the mites (hatched, nonsymptomatic; horizontal lines, symptomatic). Only diseased tissue contained the associated VLPs (*18*).

Department of Plant Pathology, Montana State University, Bozeman, MT 59717.

Table 1. The response of healthy barley seedlings that were exposed to mites collected in 1986.

Cultivar	Mite number*		
	250	100	50
Kearney	9/26†	8/15	3/6
Tennessee winter	8/26	1/9	0/5
Total	17/52	9/24	3/11

*Approximate number of mites that were initially introduced. †The numerator is the number of symptomatic plants, and the denominator is the total number of plants.

group was placed on detached healthy leaves for 13 days. We then placed one set of eight healthy barley seedlings in each cage for 1 week and then added another set of healthy seedlings to each cage for an additional 3 weeks, thus allowing one set of seedlings a 4-week inoculation access period and the other set of healthy seedlings a 3-week inoculation period. We fumigated the test plants to kill any remaining mites, then transplanted and grew them in the greenhouse.

Diapausal eggs of *P. latens* (*15*) were gathered from two sites (one in Canada and one in Montana) that previously had diseased barley during the 1986 growing season. To induce hatching, the eggs were kept moist with distilled water for at least 4 days, with initial larval emergence at day 6. The mites collected in Canada were fed on detached healthy barley leaves for about 6 days; the mites from Montana were immediately allowed to feed on healthy test seedlings. Both mite populations were allowed access to four consecutive sets of test seedlings. About 60 mites were placed into each cage containing the first set of test plants (*16*). Plants were assayed for the presence of VLPs in crude leaf extracts or ultrathin sections (*1*).

Diagnostic symptoms of the disease developed on 33% (29 in 87 plants) (Table 1) and 63% (40 in 64 plants) (Fig. 1) of the test plants that had been exposed to mites and their progeny collected from diseased barley in 1986 and 1987, respectively. In contrast, those barley seedlings that had been exposed to the mite population that originated from wheat in 1987 were nearly free of the disease (Fig. 1).

The 16 indicator test plants that we exposed to mites that had fed on detached healthy leaves produced no diagnostic symptoms, whereas 94% (15 in 16 plants) of the plants exposed to the mites that had fed on detached diseased leaves produced symptoms of the disease. VLPs were only seen in diseased leaves.

A high incidence of symptomatic plants occurred in all sets of test plants that had been exposed to the two mite populations derived from diapausal eggs (Fig. 2). Because the first generation of these mites was introduced immediately upon hatching to only healthy plant tissue and was therefore without a source of the assumed pathogenic agent, we surmised that the original inoculum came from the diapausal eggs. This is evidence for transovarial passage of the causal agent, and thus the diapausal egg may serve as an overwintering host for the agent.

The VLPs were always located intracellularly and accumulated predominately in the mesophyll cells, although VLPs were also seen in xylem, phloem, and epidermal cells of leaf tissue. In addition, VLPs were found in root, sheath, and awn tissue in affected plants.

The gross morphology of the VLPs (Fig. 3) resembled that of viruses in the Rhabdoviridae and Baculoviridae (*2*) in that all are tubular-like and usually enveloped with a membrane. However, these viruses are shorter and some have cross-striations. Several different unclassified viruses infecting mammals (*5*) and insects (*6*) consist of long, tubular-like particles that may or may not be enclosed by a membrane.

Because viruses that most resemble these unusual VLPs in barley belong to the animal kingdom, perhaps the VLPs originated from the vector responsible in spreading the

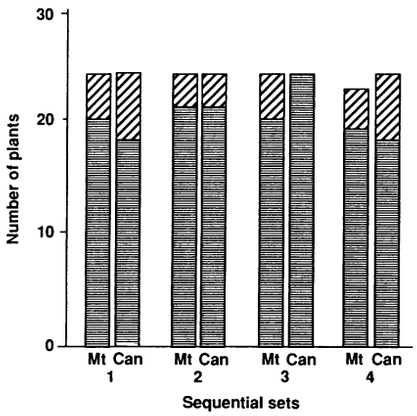

Fig. 2. Effects of exposing healthy barley seedlings to mites that were initially hatched from diapausal eggs (the progeny were derived from active eggs). The diapausal eggs from diseased sites in Canada (Can) and Montana (Mt) produced mites that caused symptoms (horizontal lines) in plants and the occurrence of the unique VLPs within the tissue. Nonsymptomatic plants (hatched) had mite feeding damage but never expressed disease symptoms typical of the disease or revealed the presence of VLPs.

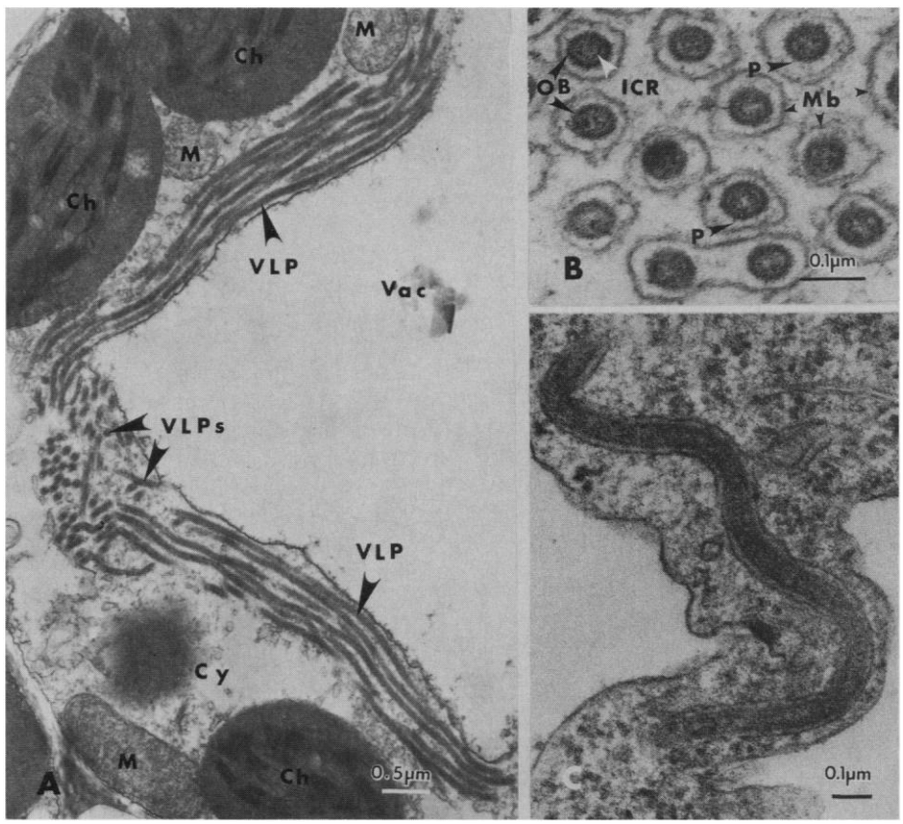

Fig. 3. Transmission electron micrographs depicting VLPs in the cytoplasm (Cy) of diseased barley leaf in mesophyll cells. (**A**) A cluster of long flexuous VLPs (Ch, chloroplast; M, mitochondrion; Vac, vacuole). (**B**) Cross sections of the VLPs revealing a loose membrane (Mb), apparent projections (P), and an outer band (OB) enclosing an inner circular ring (ICR) of material. (**C**) A longitudinal view of a magnified VLP.

assumed causal agent to the plants. Therefore, the brown wheat mite may play the role as both a vector and a host for the VLPs. Indeed, electron microscopy of ultrathin sections of infective mites indicates the presence of morphologically similar VLPs in the gut (*17*).

Petrobia latens is a dry-weather pest and is usually a threat only when a crop is under drought stress (*11*). Coincidentally, from 1983 through 1986, north central Montana experienced the most damaging drought conditions since the 1930s. Also, barley acreage has increased considerably during the 1980s, thereby creating an abundant supply of host plants for both the causal agent and its vector. Changes in climate and vegetation most likely contributed to the epidemic levels of the disease.

REFERENCES AND NOTES

1. Diseased and healthy barley tissue were fixed first in 3% glutaraldehyde and then in 2% osmium tetroxide, dehydrated in a graded ethanol series, and embedded in Spurr's epoxy resin [A. R. Spurr, *J. Ultrastruct. Res.* **26**, 31 (1969)]. Sections were double stained with uranyl acetate and lead citrate [E. S. Reynolds, *J. Cell Biol.* **17**, 208 (1963)]. Crude leaf extracts were prepared by grinding tissue in 0.05*M* tris-HCl buffer, *p*H 7.2, and placing the diluted leaf sap onto parlodian-coated grids followed by uranyl acetate staining.
2. In sap preparations (in vitro) the widths of some of the particles varied considerably (between 41 and 74 nm) along their lengths but most particles were uniformly 50 to 60 nm in diameter.
3. R. E. F. Matthews, *Intervirology* **17**, 1 (1982).
4. B. Kassanis, *Phytopathol. Z.* **111**, 363 (1984).
5. M. P. Kiley *et al.*, *Intervirology* **18**, 24 (1982).
6. M. K. Arnold and G. Barson, *J. Invertebr. Pathol.* **29**, 373 (1977); L. Bailey, J. M. Carpenter, R. D. Woods, *Virology* **114**, 1 (1981); T. B. Clark, *J. Invertebr. Pathol.* **32**, 332 (1978); M. O. Odindo, C. C. Payne, N. E. Crook, P. Jarett, *J. Gen. Virol.* **67**, 527 (1986); P. J. Krell, *ibid.* **68**, 1477 (1987).
7. N. L. Robertson, thesis, Montana State University, Bozeman (1987).
8. R. E. F. Matthews, *Plant Virology* (Academic Press, New York, 1981), pp. 568–607.
9. D. M. Tuttle and E. W. Baker, *Spider Mites of Southwestern United States and a Revision of the Family Tetranychidae* (Univ. of Arizona Press, Tucson, 1968), pp. 51–56.
10. The brown wheat mite has eight distinct stages of growth and maturation, which include the egg, larva, two sequential nymphal stages, and the adult; quiescent (chrysalis) stages occur after the larval and each of the nymphal stages. Depending on the environment, the transition from larva to adult may take as little as 7 days or as long as 20 days. The adult may live up to a month before it becomes moribund and dies. F. A. Fenton, *J. Econ. Entomol.* **44**, 996 (1951); R. M. Khan, S. L. Doval, H. C. Joshi, *Indian J. Entomol.* **31**, 258 (1969); T. Lu, *Acta Entomologica Sinua* **22**, 477 (1979); J. M. Del Rivero and F. G. Mari, *Bol. Serv. Plagas. For.* **9**, 109 (1983).
11. H. C. Cox and F. V. Lieberman, *J. Econ. Entomol.* **53**, 704 (1960).
12. H. T. Reynolds, *ibid.* **44**, 642 (1951); G. S. Deoul, *Indian J. Agric. Sci.* **44**, 681 (1974).
13. In the laboratory, the mites were identified and separated from other arthropods under a light microscope by anesthetizing the field-collected specimens; active mites were placed on healthy test plants (one- to two-leaf stage).
14. The top 2 cm of soil from each pot of infested test plants was removed and then used as the top soil layer in the new set of plants. This was done because some mites and their active red eggs are found in the soil, and thereby the number of mites was maximized for each set of plants.
15. An adult mite may only lay one type of egg, which includes either a diapausal or an active egg. The red, spherical, active egg hatches in about 10 days or will become nonviable. The white, diapausal egg must have at least 2 months of dormancy before it hatches, and then only if moisture is present and the temperature is relatively warm [A. D. Lees, *J. Insect Physiol.* **6**, 146 (1960)].
16. The test plants exposed to the mites from Canada were grown under a 12-hour-per-day photoperiod with metal halide lamps (40,000 lux); the plants exposed to mites from Montana were under a 16-hour-per-day photoperiod with natural daylight supplemented with halide lamps. Temperatures were between 22° and 27°C.
17. Four VLPs that resemble those VLPs found in barley tissue have been identified in the gut of *P. latens*. However, these VLPs could have been ingested by the mite and retained in the gut instead of passing out through the stylets during subsequent feeding.
18. Additional information was obtained for distinguishing symptoms caused by mite feeding and those induced by the putative causal agent. Infested leaves usually had trails of whitish to silvery dots, which eventually developed into a silvery or pale yellow overcast if the mite numbers were great. Often, the leaf margins had a distinct outline of whitish-yellow from mite feeding. In contrast, those plants that had VLPs also had leaves with a mosaic of light green to yellow dashes and streaks; the plants exposed to the noninfective mites never produced diagnostic streaks and stripes. The symptoms continued to develop on newly emerging leaves of affected plants after the mites had been removed from the plants. This implies that the infective mites may have been responsible for transmitting a material into the plants that is translocated, inducing the discoloration. The consistent presence of the VLPs only in diseased tissue suggests that the VLPs are being transmitted into the plants by the mites. Alternatively, the mites could transmit a yet unidentified agent to the host plant, which in turn induces the formation of the VLPs. However, such structures have not been identified in plant cells.

19. We thank J. R. Baringer for supplying producer contacts and J. R. Craig for technical assistance. Supported by Montana Agricultural Experiment Station project MONB00225 and Montana Wheat and Barley Committee grant 2-6000-561. Journal Series Paper J2088 Montana Agricultural Experiment Station, Bozeman.

24 December 1987; accepted 1 April 1988

Macroevolutionary Interpretations of Symmetry and Synchroneity in the Fossil Record

JENNIFER A. KITCHELL AND NORMAN MACLEOD

Quantitative analyses of global diversity in the marine fossil record over Phanerozoic time reveal an historically ordered pattern of sequential dominance and increasing diversity. Explanatory models applied to this empirical pattern lead to irreconcilable differences of interpretation. The issue may be resolved by determining the expected distributions and limits of temporal covariation among clades generated by a random branching process. Results also challenge the claim that asymmetries in intra-clade diversity variation provide a directional arrow for the history of life.

IN THE LAST DECADE, THE ISSUE OF whether the global pattern of total diversity change of the marine fossil record over Phanerozoic time has been steady state (equilibrium) or directional has been decided in favor of directional increase with time (*1*). Within this overall trend, a series of studies (*2*) has recognized three groups of taxa, or so-called evolutionary faunas, that vary in diversity more or less synchronously through time and whose dominance, once relinquished, is not regained: "Each of the three major faunas seems to have its own characteristic diversity so that its expansion or contraction appears as being intimately associated with a particular phase in the history of total marine diversity" (*3*). In a related analysis of within-clade diversity, Gould *et al.* (*4*) proposed a measure of temporal directionality based on the asymmetry of clade shape and concluded that directionality was dominant in the early Phanerozoic. They stated their intention "to replace the grand, but vague and noisome notion of progress with a question. . . imbued with the twin virtues of definition and testability: if you were handed a chart of clade diversity diagrams with unlabeled axes, would you know whether you were holding the chart upside down or right side up?"

A current dilemma in analyzing both clade symmetry and diversity, admitted to by Gould *et al.* (*4*) and the focus of a current controversy (*5*) involving the interpretation of patterns within the data compiled by Sepkoski (*3*), is that the taxonomic framework on which these data sets are based is largely paraphyletic. For evolutionary analyses, paraphyletic groupings are considered to be invalid in that they do not contain all descendants. Rather, they represent a portion of a monophyletic group based on an arbitrarily chosen feature. Patterns in paraphyletic groups consequently will vary depending on the criterion used to form them. Properly defined evolutionary clades are

Department of Geological Sciences and Museum of Paleontology, University of Michigan, Ann Arbor, MI 48109.