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Influence of Storage, Season, Morphology and Habitat on Haemagglutinin and Haemolysin Levels in Maritime Lichens

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Abstract

Relatively non-specific haemagglutinins (HGN) and haemolysins (HLY), ranging in titre from 2^{-1} to 2^{-9} and 2^{-1} to 2^{-7} respectively, were detected in maritime woodland and sea shore lichens. Mean HGN and HLY levels, and individual titres, were overall higher in crustose and foliose compared to fruticose species. Significant intermorphological differences between titres were not found, except with the use of human and sheep erythrocytes, when the three thallus types were compared with each other. Both intrageneric and intergeneric differences in HGN and HLY levels were found for each of the morphological forms. Titre variations were especially detected between certain species within the genera *Parmelia* and *Lecanora*. Crustose and foliose lichens displayed the highest degrees of dissimilarity in titres whereas similar titre relationships occurred between fruticose and either crustose or foliose forms. *Lecanora atra* and *Rhizocarpon geographicum* exhibited the least relatedness in titres whilst all the fruticose lichens examined shared close relationships between titre values. Fresh, unfrozen and unstored lichen extracts displayed little differences in titre endpoints when compared to frozen and stored material whereas samples, in particular foliose species, collected in autumn possessed slightly higher amounts of HGN but not HLY against certain erythrocyte types than those obtained in summer. Sea shore lichens contained higher levels of HLY than the corresponding woodland species whilst the converse was true for HGN levels.

Keywords: maritime lichens, crustose, foliose, fruticose, haemagglutinins, haemolysins, storage, morphology, season, habitat

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1. Introduction

Agglutinins (lectins) and lysins, both specific and non-specific, have been demonstrated against human and animal erythrocytes in angiosperms and bacteria (Liener et al., 1986), algae (Ingram, 1985a; 1985b), fungi (Nordbring-Hertz and Chet, 1986) and mainly terrestrial lichens (Barrett and Howe, 1968; Estola and Vartia, 1955; Hardman et al., 1983). Maritime lichen extracts have been investigated by Rogers et al. (1980) and Ingram (1982). The latter author found that they possessed natural, relatively non-specific HGN and HLY whose activities were dependent upon assay incubation temperatures. However, only limited information is available concerning the influence of sampling site (Seeger et al., 1973), climate (Seeger and Wiedmann, 1972) and other environmental factors (Ingram and Tassabehji, 1988a) on levels and presence of HGN and HLY in lower plants.

To the author's knowledge there are no reports concerning the potential effects of habitat, time of year or thallus morphology on the amounts of such substances in lichens. On exposed sea-shore rocks found along the coast of south-west Wales, lichens with the crustose morphology dominate with relatively few species of foliose and fruticose forms (Ferry and Sheard, 1969) whereas the converse is true for sheltered woodland tree lichens in the same area (Ferry, 1971). These two habitats represent different environmental conditions which would affect both lichen physiology and biochemistry of metabolism (Brodo, 1973; Fletcher, 1980).

This paper reports the findings of further analyses of the results obtained in a previous study on stored, frozen extracts of maritime lichens collected in early July (Ingram, 1984) when compared to the HGN and HLY titres, determined in the present work, with specimens obtained in late September. The autumn-collected samples were analysed for the presence of HGN and HLY using both fresh, unstored and unfrozen extracts and those which had been kept under frozen storage. Summer collected lichens, including those species that gave negative results in the author's initial study (Ingram, 1982), were re-examined using fresh, unfrozen and unstored preparations for naturally-occurring anti-erythrocyte agglutinins and lysins. This experiment was performed to ascertain whether freezing and thawing of the lichen extracts were responsible for the lack of or reduced agglutination and/or lytic activities. The effects of lichen morphology, together with the influence of woodland and sea shore habitats, on the levels of HGN and HLY in lichens were investigated. Inter- and intrageneric, and inter- and intramorphological type, comparisons of titres were also performed.

2. Materials and Methods

Specimen sampling

Lichens were collected in autumn from maritime woodland trees and from rocks in the littoral, supralittoral and terrestrial zones of the sea-shore in and around the Dale Peninsula, southwest Wales.

The species sampled in the current work were *Lecidea subincongrua* Nyl., *Pertusaria pseudocorallina* (Liljebl) Arnold., *Lecanora atra* (Huds.) Ach., *L. helicopis* (Wahlenb. ex Ach.) Ach., *L. actophila* Wedd., *Rhizocarpon geographicum* (L.) DC., *R. constrictum* Malme., *Buellia chlorophaea* (Hepp ex Leight.) Lett., *Ochrolechia parella* (L.) Massal., *Caloplaca marina* (Wedd.) Zahlbr., *C. thallincola* (Wedd.) Du Rietz., *Candelariella vitellina* (Hoffm.) Mull. Arg. *Lecania erysibe* (Ach.) Mudd., *Lepraria incana* (L.) Ach., *Anaptychia fusca* (Huds.) Vain., *Xanthoria parietina* (L.) Th. Fr., *Parmelia prolixa* (Ach.) Carroll., *P. loxodes* Nyl., *P. aspera* Massal., *P. caperata* (L.) Ach., *P. perlata* (Huds.) Ach., *P. saxatilis* (L.) Ach., *P. sulcata* T. Taylor., *P. glabratula* (Lamy) Nyl., *P. subaurifera* Nyl., *P. subrudecta* Nyl., *Physcia aipolia* (Ehrh. ex Humb.) Hampe., *Cladonia caespiticia* (Pers.) Florke., *C. pyxidata* (L.) Hoffm., *Ramalina siliquosa* (Huds.) A.L. Sm., *R. farinacea* (L.) Ach., *R. fastigiata* (Pers.) Ach., *Evernia prunastri* (L.) Ach., *Usnea fragilescens* Hav. ex Lynge., *Lichina confinis* (O.F. Mull.) C. Ag. and *L. pygmaea* (Lightf.) C. Ag.

Identification, extraction and assay

The identification of the maritime lichens, production of lichen extracts, erythrocyte preparation and details of the haemagglutination and haemolysin tests have been reported before (Ingram and Tassabehji, 1988a). Erythrocytes were obtained from trout, salmon, frog, lizard, goose, chicken, pigeon, turkey, donkey, goat, horse, sheep, rabbit, rat, mouse, calf and human groups A, B, AB and O. In the present work, the temperatures of incubation for the determination of haemolytic and haemagglutination endpoint titres were 21°C and 37° respectively.

The assays were replicated for all samples examined using different batches of extract and erythrocytes on each occasion. In the effect of habitat study, the assays were conducted in triplicate with the use of chicken erythrocytes because these cells gave positive results with all of the chosen lichen species.

Statistical treatment of results

The results were analysed by one-way analysis of variance and correspondence analysis. coefficients of dissimilarity between titres and erythrocytes for each lichen species were calculated as a basis for cluster analysis. Correspondence analysis is an ordination method, related to principal component analysis, but appropriate for discrete rather than continuous variables. In ordination the titres of similar samples are grouped together; two ordinations are plotted, one of sample (titres) and one of variables (erythrocytes) and vice versa, and sample scores are weighted averages of the variables. This technique perfects the correspondence of variables and the sample scores were used to give diagrammatic reproduction of the multivariate data set. It can give a good 2-dimensional view of the data provided the percentage of total variation accounted for by the first two axes is appreciable. The above procedure was used to see whether there were any HGN or HLY titre relationships between lichens both intra- and intermorphologically, intra- and intergenerically, and between the various erythrocyte types.

3. Results

Effect of storage and season

The re-examination of 36 autumn-collected lichen species using stored, frozen and unstored, unfrozen extracts resulted in either single endpoint dilution differences or no changes in either HGN or HLY titres. Furthermore endpoint values in most cases were similar to those determined in a previous study (Ingram, 1984). However both unfrozen, unstored and frozen, stored extracts of several of the foliose *Parmelia* species gave slightly raised HGN and HLY levels compared to those collected in summer with differences in titres of up to two endpoint dilutions. Moreover, autumn collected *A. fusca* and *P. caperata; L. incana*, and *C. caespiticia* and *R. siliquosa* (all previously unreactive) agglutinated human erythrocytes from blood groups AB, O and B respectively albeit to a low titre of 2^{-1}. Likewise, HGN titres of up to 2^{-2} were currently detected in *O. parella, R. siliquosa, X. parietina* and *P. saxatilis* against pigeon erythrocytes; and in *C. thallincola* and *L. incana*, and *P. perlata* and *P. sulcata*, against turkey and goose erythrocytes respectively. In addition, of the crustose lichens previously negative for HGN and HLY namely (*L. pygmaea, L. confinis, L. subincongrua, L. erysibe, C. vitellina, P. pseudocorallina* and *R. constrictum*), the undiluted extract of *C. vitellina* weakly agglutinated erythrocytes from chicken, horse and rabbit. No changes were detected with regard to extracts previously negative for HLY.

Influence of morphology type

The means, standard errors of the means (s.e.m.) and ranges of HGN and HLY titres of the lichen extracts together with the number of positive erythrocyte types tested are given in Table 1.

HGN titres of generally 2^{-5} or less were detected in the 29 reactive species tested against many of the positive erythrocyte types. HGN levels of 2^{-6} were found with *A. fusca* and *R. geographicum* (against goat erythrocytes), *B. chlorophaea* (chicken), *P. caperata* (goose, pigeon and calf) and *U. fragilescens* (rabbit). The highest HGN titres ranged from 2^{-7} to 2^{-9} and were observed in certain crustose and foliose lichens viz. *L. atra* (chicken and frog), *L. helicopis* (sheep), *O. parella, P. glabratula* and *C. marina* (chicken), *C. thallincola* (rabbit), *L. incana* (lizard, chicken and calf), *P. perlata* (chicken, frog, pigeon and rabbit), *P. saxatilis* (horse) and *P. sulcata* (frog and horse). The maximum HGN titre of 2^{-6} obtained for fruticose lichens was found with *U. fragilescens* against rabbit erythrocytes.

HLY titres ranging from 2^{-1} to 2^{-4} were demonstrated in most cases with positive extracts irrespective of morphological form. However, higher HLY activity was detected in *L. incana* and *P. glabratula* (2^{-5}), *A. fusca* (2^{-6}) and *X. parietina* (2^{-7}) against chicken and rabbit, goat and horse erythrocytes respectively. Over 55% of the lichen species examined caused the agglutination of frog, goose, chicken, goat, horse, sheep and rabbit erythrocytes, and also lysis of the above erythrocyte types (except for frog and sheep). Mouse erythrocytes were only agglutinated exclusively by *U. fragilescens* extracts. The levels of HGN were in general higher than those of the HLY.

Statistical analysis

Data with mouse and rat erythrocytes were omitted due to their poor reactivity with the lichen extracts. To determine whether there were any differences in titres with regard to morphology, data for each of the three thallus types were separately bulked together for all positive erythrocyte types (see Tables 2 and 3). The mean $\pm$s.e.m. (N or total number of positive erythrocytes) HGN titre ($\log_2$) for crustose lichens was 1.95 ± 0.74 (N = 16), for foliose species 1.42 ± 0.47 (17) and for fruticose forms 1.22 ± 0.61 (18). In the case of HLY, the calculated values were 0.93 ± 0.34 (17), 0.92 ± 0.26 (18) and 0.74 ± 0.39 (18) respectively. Comparisons of both mean and individual HGN and HLY levels between the three forms revealed no significant differences ($P>0.05$) even though crustose forms displayed overall the highest, foliose intermediate and fruticose the lowest titres. Nevertheless, a comparison of

Table 1. [x]Mean haemagglutinin and haemolysin titres (log_2) ± standard errors of the means (ranges) of woodland and sea shore crustose, foliose and fruticose lichens against twenty erythrocyte types from selected vertebrates. [xx]The total number of positive erythrocyte types for each lichen species is also indicated.

	Haemagglutinin			Haemolysin		
Crustose						
*** *Lecanora atra*	[x]4.2±0.6	(1–7)[xx]	10	2.3±0.4	(1–4)	6
* *L. helicopis*	4.3±0.6	(3–7)	6	2.0±0.4	(1–3)	6
* *L. actophila*	3.2±0.2	(3–4)	5	1.8±0.7	(1–3)	8
*** *Rhizocarpon geographicum*	3.9±0.5	(2–6)	7	2.6±0.4	(1–4)	9
*** *Buellia chlorophaea*	4.0±1.0	(3–6)	3	1.4±0.2	(1–2)	5
*** *Ochrolechia parella*	4.0±0.7	(1–8)	10	2.2±0.4	(1–3)	9
* *Caloplaca marina*	3.6±0.7	(1–7)	9	1.6±0.2	(1–3)	10
* *C. thallincola*	3.8±0.5	(1–7)	11	1.9±0.3	(1–3)	10
** *Lepraria incana*	4.8±0.6	(1–7)	13	2.4±0.4	(1–5)	14
Foliose						
*** *Anaptychia fusca*	3.3±0.7	(1–6)	6	3.0±0.6	(1–6)	9
*** *Xanthoria parietina*	2.5±0.4	(1–4)	10	2.9±0.6	(1–7)	10
** *Physcia aipolia*	1.5±0.5	(1–2)	2	1.5±0.3	(1–2)	4
** *Parmelia proliza*	2.9±0.4	(1–5)	8	1.8±0.2	(1–3)	10
* *P. loxodes*	2.8±0.5	(2–5)	6	1.2±0.1	(1–2)	9
** *P. aspera*	4.0±0.6	(3–5)	3	1.3±0.2	(1–2)	6
** *P. caperata*	4.3±0.4	(1–6)	14	2.1±0.3	(1–4)	15
** *P. perlata*	5.9±0.5	(3–9)	11	2.3±0.3	(1–4)	11
*** *P. saxatilis*	3.8±1.2	(1–8)	5	1.2±0.1	(1–2)	10
** *P. sulcata*	3.9±0.6	(1–8)	11	1.9±0.3	(1–3)	11
** *P. glabratula*	3.5±0.8	(1–7)	8	2.1±0.4	(1–5)	10
** *P. subaurifera*	3.5±1.3	(3–4)	2	1.6±0.4	(1–3)	5
** *P. subrudecta*	3.5±0.6	(2–5)	4	1.5±0.3	(1–3)	8
** *Cladonia caespiticia*	3.3±0.5	(1–5)	8	1.7±0.3	(1–3)	6

Fruticose						
*** *Ramalina siliquosa*	2.4±0.3	(1–4)	13	1.3±0.2	(1–2)	10
** *R. farinacea*	3.0±0.7	(2–5)	4	1.2±0.2	(1–2)	5
** *R. fastigiata*	2.3±0.5	(1–4)	7	1.7±0.3	(1–3)	6
** *Evernia prunastri*	2.0±0.7	(1–4)	4	1.3±0.3	(1–2)	4
** *Usnea fragilescens*	2.8±0.4	(1–6)	17	2.0±0.2	(1–4)	18
*** *Cladonia pyxidata*	3.3±0.5	(1–5)	7	1.3±0.2	(1–2)	7

* Rocky sea-shore or **woodland lichens only.

***Lichens present on both sea shore rocks and woodland trees.

titres determined with individual erythrocyte types with bulked data for each of the morphological forms revealed a significant ($P<0.05$) difference in both HLY and HGN values between foliose and fruticose forms against human AB erythrocytes. HLY levels were significantly different ($P<0.05$) between crustose and foliose lichens when sheep and human O erythrocytes were used.

Inter- and intramorphological, and intergeneric comparisons

Within the crustose, foliose and fruticose groups, the highest degrees of intramorphological and intergeneric variation and hence unrelatedness of HLY titres were found between *L. atra* and *R. geographicum, P. loxodes* and *C. caespiticia*, and *R. siliquosa* and *R. fastigiata* with dissimilarity coefficients of 29.6, 6.0 and 4.0 respectively. The least relationships were found between the crustose species *R. geographicum* and *C. thallincola*, and the foliose *P. loxodes* and *P. subrudecta*, and *P. prolixa* and *P. sulcata* (all with coefficients of 3.0).

When the three morphological forms were compared with each other, the greatest degrees of dissimilarity occurred between *R. geographicum* and *P. loxodes* (coefficient 17.4), *L. atra* and *R. siliquosa* (10.7), and *P. aspera* and *E. prunastri* (2.0). By comparison, the most similarities in HLY levels, all with a coefficient of 2.0, were found between *L. incana* and *U. fragilescens; L. helicopis* and *P. aspera*; and *L. actophila* and *P. glabratula*. Most of the remaining comparisons gave coefficient values of 3.0 to 9.0, indicative of certain degrees of relatedness, as shown by similar grouping patterns of HLY titres in the correspondence analysis attributes ordination plot in Fig. 1 and similar titre ranges (Table 1). Exceptions to this appear to be *B. chlorophaea,*

Table 2. *Mean haemolysin titres (log_2)± standard errors of the means (ranges) of extracts of different lichen morphological forms against selected vertebrate erythrocyte types. Numbers of positive extracts (**), and lichen species used (n).

Erythrocyte type	Lichen morphology Crustose (n=9)			Foliose (n=14)			Fruticose (n=6)		
Trout	*0.4±0.3	(2–2)	**2	0.07±0.1	(1–1)	1	0.20±0.2	(1–1)	1
Salmon	0.8±0.4	(2–3)	3	0.07±0.1	(1–1)	1	ND		
Frog	0.9±0.6	(4–4)	2	1.2±0.3	(1–4)	9	1.0±0.4	(1–2)	3
Lizard	1.1±0.6	(3–4)	3	1.0±0.4	(1–3)	9	0.2±0.2	(1–1)	1
Goose	0.4±0.2	(1–2)	3	1.7±0.4	(1–5)	11	0.8±0.6	(1–3)	2
Chicken	2.4±0.5	(1–5)	8	2.3±0.4	(1–5)	12	1.2±0.7	(1–4)	3
Pigeon	1.1±0.4	(1–3)	6	1.1±0.3	(1–3)	10	0.8±0.5	(2–2)	2
Turkey	0.8±0.4	(1–3)	4	0.3±0.3	(1–3)	2	0.4±0.4	(2–2)	2
Donkey	1.4±0.4	(1–3)	6	0.6±0.3	(1–3)	4	0.4±0.2	(1–1)	2
Goat	1.0±0.4	(1–3)	6	0.8±0.4	(1–6)	5	0.6±0.6	(3–3)	1
Horse	0.8±0.2	(1–2)	5	1.5±0.5	(1–7)	10	1.4±0.5	(1–3)	4
Sheep	0.4±0.2	(1–2)	4	1.1±0.3	(1–3)	8	0.8±0.2	(1–1)	4
Rabbit	2.3±0.4	(1–5)	9	1.7±0.3	(1–4)	13	1.0±0.3	(1–2)	4
Rat	ND			ND			0.2±0.2	(1–1)	1
Mouse		ND		0.7±0.1	(1–1)	1	0.2±0.2	(1–1)	1
Calf	0.4±0.4	(4–4)	1	0.4±0.2	(1–2)	5	0.2±0.2	(1–1)	1
Human 0	1.0±0.4	(1–3)	4	1.3±0.3	(1–3)	11	1.6±0.7	(2–3)	3
Human A	0.4±0.2	(1–1)	4	0.2±0.1	(1–1)	3	1.0±0.4	(1–2)	3
Human B	0.3±0.2	(1–2)	2	0.4±0.1	(1–1)	6	0.8±0.4	(1–2)	3
Human AB	1.0±0.4	(1–3)	5	1.1±0.2	(1–3)	10	1.2±0.5	(2–2)	3

ND = Not Detected

R. geographicum, A. fusca, L. atra, O. parella, R. fastigiata and *C. thallincola* which lie outside of the main clumping pattern areas, more so with the last four species (Fig. 1), thus indicating little similarity to each other and to the rest of the lichens examined with regard to HLY titres.

In consideration of HGN, the most dissimilarity in titres occurred between the crustose species *L. atra* and either *R. geographicum* (coefficient 33.1), *L. helicopis* (17.7) or *L. incana* (13.2), and between *R. geographicum* and *B. chlorophaea* (20.9). All the fruticose forms when compared with each

Table 3. *Mean haemagglutinin titres (log_2) ± standard errors of the means (ranges) of extracts of different lichen morphological forms against selected vertebrate erythrocyte types. Numbers of positive extracts **, and lichen species used (n).

Erythrocyte type	Lichen morphology Crustose (n=9)			Foliose (n=14)			Fruticose (n=6)		
Trout	*0.8±0.5	(1–4)	**3	0.9±0.1	(1–4)	5	0.8±0.5	(2–2)	2
Salmon	1.1±0.6	(3–4)	3	ND			ND		
Frog	1.4±0.9	(6–7)	2	1.8±0.7	(1–8)	7	1.6±0.8	(2–3)	3
Lizard	1.9±0.8	(3–7)	4	1.1±0.5	(1–5)	5	0.2±0.2	(1–4)	2
Goose	1.6±0.7	(1–5)	5	2.3±0.5	(2–6)	9	1.4±0.7	(1–3)	3
Chicken	4.9±1.1	(3–8)	7	3.0±0.7	(1–9)	10	1.8±0.8	(2–4)	3
Pigeon	2.3±0.8	(3–5)	5	1.3±0.6	(1–7)	5	1.4±1.0	(2–5)	2
Turkey	0.6±0.4	(2–3)	2	0.2±0.2	(3–3)	1	1.0±0.8	(1–4)	2
Donkey	1.8±0.7	(3–4)	3	0.9±0.5	(3–6)	3	0.8±0.6	(1–3)	2
Goat	2.4±0.9	(1–6)	6	1.5±0.5	(2–6)	6	0.6±0.2	(1–1)	3
Horse	2.4±0.5	(2–4)	7	2.6±0.7	(2–8)	9	1.0±0.6	(2–2)	1
Sheep	1.6±0.9	(2–7)	3	1.3±0.5	(1–6)	7	2.2±0.7	(1–4)	4
Rabbit	4.0±0.8	(4–7)	7	2.1±0.6	(1–7)	8	3.6±1.0	(3–6)	4
Rat	ND			ND			0.2±0.2	(1–1)	1
Mouse	ND			0.1±0.10	(1–1)	1	0.2±0.2	(1–1)	1
Calf	1.0±0.8	(1–7)	3	1.1±0.5	(3–6)	4	0.6±0.4	(1–2)	2
Human 0	2.4±0.6	(3–4)	6	2.5±0.6	(1–5)	10	1.8±0.8	(2–4)	3
Human A	ND			0.1±0.1	(2–2)	1	1.4±0.9	(3–4)	2
Human B	1.1±0.8	(4–6)	2	0.8±0.5	(1–4)	5	0.6±0.4	(1–2)	2
Human AB	1.8±0.8	(3–6)	4	1.9±0.5	(3–5)	8	1.8±0.8	(2–4)	3

ND = Not Detected

other exhibited closely related titres whilst the foliose species, *A. fusca* and *P. saxatilis* (7.0) were the most dissimilar and *P. subaurifera* and *P. aspera* (0) the most similar.

When intergeneric comparisons of HGN titres were made between the three morphological forms, the highest degrees of similarity occurred between *B. chlorophaea* and *U. fragilescens* (2.0), *R. geographicum* and *C. caespiticia* (3.0), and *R. fastigiata* and *P. subrudecta* (5.0) whilst the least relationships were found between *B. chlorophaea* and either *P. loxodes* or *A. fusca*,

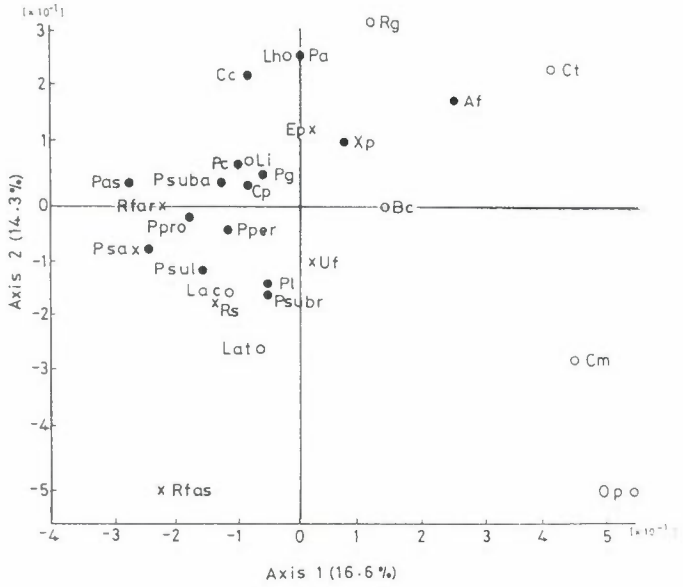

Figure 1. Correspondence analysis ordination of lichen species based on their haemolysin titers against various erythrocyte types. *Lecanora atra* (Lat), *Lecanora helicopis* (Lh), *Lecanora actophila* (Lac), *Rhizocarpon geographicum* (Rg), *Buellia chlorophaea* (Bc), *Ochrolechia parella* (Op), *Caloplaca marina* (Cm), *Caloplaca thallincola* (Ct), *Lepraria incana* (Li), *Anaptychia fusca* (Af), *Xanthoria parietina* (Xp), *Parmelia proliza* (Ppro), *Parmelia loxodes.* (Pl), *Parmelia aspera* (Pas), *Parmelia caperata* (Pc), *Parmelia perlata* (Pper), *Parmelia sulcata* (Psul), *Parmelia saxatilis* (Psax), *Parmelia glabratula* (Pg), *Parmelia subaurifera* (Psuba), *Parmelia subrudecta* (Psubr), *Physcia aipolia* (Pa), *Cladonia caespiticia* (Cc), *Cladonia pyxidata* (Cp), *Ramalina siliquosa* (Rs), *Ramalina farinacea* (Rfar), *Ramalina fastigiata* (Rfas), *Evernia prunastri* (Ep), *Usnea fragilescens* (Uf).
Foliose •; Fruticose X; Crustose O lichens

and between *P. saxatilis* and *R. farinacea* with coefficients of 15.6, 8.9 and 5.0 respectively. The majority of both inter- and intramorphological, and intergeneric comparisons gave dissimilarity coefficient values over a range of 2.0 and 9.0 and many of the lichens were scattered along and in relatively close proximity to the horizontal axis of the ordination plot (Fig. 2). However, *R. fastigiata, P. subrudecta* and *P. loxodes* were noticeably removed from the rest of the lichens with regards to their HGN activities whereas eleven of the species (*C. marina, B. chlorophaea, C. thallincola, E. prunastri, R. siliquosa, C. pyxidata, P. aspera, A. fusca, O. parella, L. helicopis* and *R. farinacea*) occurred, albeit separately, outside of the correspondence plot area of the horizontal axial species distribution (Fig. 2).

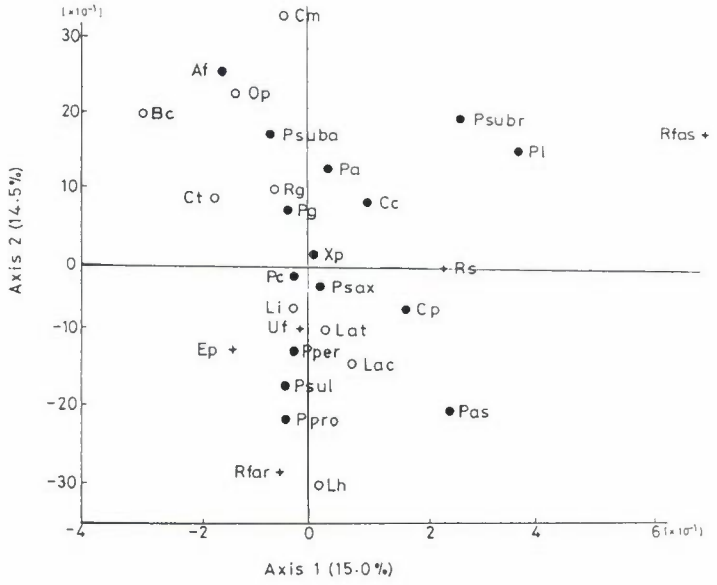

Figure 2. Correspondence analysis ordination of lichen species based on their haemagglutinin titres against various erythrocyte types. (Full names of abbreviated species as per Fig. 1).

Table 4. *Means ± standard errors of the means (ranges) of anti-chicken erythrocyte haemagglutinin and haemolysin titres (log_2) for maritime lichen species found both on sea shore (SS) and in woodland (WD) habitats.

Lichens	Haemagglutinin SS	Haemagglutinin WD	Haemolysin SS	Haemolysin WD
L. atra	*2.8±0.5 (2.3)†	6.3±0.7 (5–7)	3.5±0.5 (3–4)	1.8±0.3 (1–3)
R. geographicum	3.2±0.4 (2–4)	5.5±0.5 (5–6)	3.4±0.2 (3–4)	1.5±0.3 (1–2)
**B. chlorophaea*	3.0 †	6.0	2.0	1.0
O. parella	2.6±0.5 (2–3)‡	7.3±0.7 (6–8)	3.3±0.3 (3–4)	1.8±0.4 (1–3)
**A. fusca*	2.8±0.6 (2–4)†	6.0	5.0±0.6 (5–6)†	2.0±0.4 (2–3)
X. parietina	1.4±0.2 (1–2)	3.6±0.2 (3–4)	5.0±1.0 (4–6)†	2.0±0.4 (2–3)
**P. saxatilis*	2.8±0.8 (2–4)‡	8.8	2.0	1.0
C. pyxidata	4.3±0.3 (4–5)	4.3±0.3 (4–6)	1.5±0.2 (1–2)	2.3±0.3 (2–3)
**R. siliquosa*	2.0±0.3 (2–3)	3.5±0.5 (3–4)	2.0	1.0

**For some of the species only the mean titre has been given. This is because all the values were the same for each triplicate assay and hence there is no standard error of the mean or range

Levels of significance; †$P < 0.05$, ‡$P < 0.01$

Intrageneric comparisons

The highest degrees of intrageneric variation, in both HLY and HGN titres, were found between *L. helicopis* and *L. atra* or *L. actophila, P. loxodes* and *P. subrudecta*, and *P. prolixa* and *P. sulcata.* In addition, the most dissimilarity in HGN titres only occurred between *P. aspera* and *P. subaurifera*, and both *P. caperata* and *P. perlata* with *P. prolixa.* The least relatedness between HLY titres only was found between *R. siliquosa* and *R. fastigiata.*

Erythrocyte comparisons

When each of the erythrocyte types was considered individually, haemolysis of trout and especially turkey, salmon and human A, B an AB erythrocytes were independent of and non-associated with two groupings, each with similar HLY titres, that were distinguished from the above erythrocyte types (Fig. 3). Goat, lizard and donkey erythrocytes were clustered in one group relatively adjacent to a second group which comprised the remainder of the erythrocyte types. In the case of the HGN activities, erythrocytes from

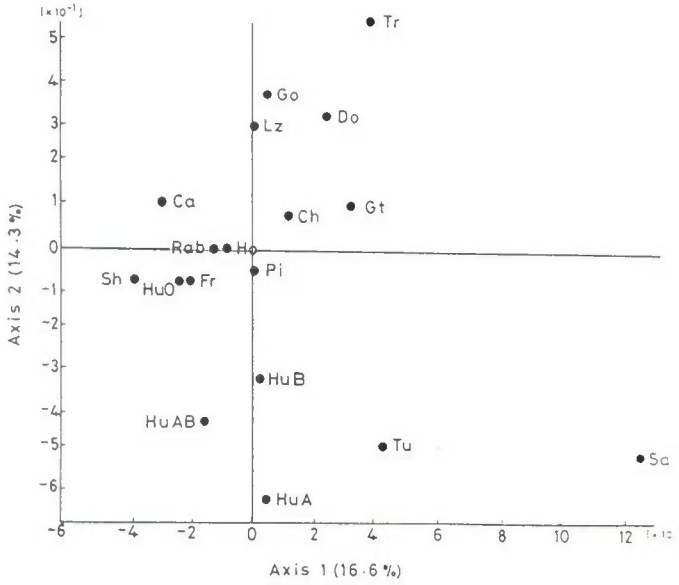

Figure 3. Corresponding ordination of erythrocyte types (•) based on haemolysin titres of the lichen species. Trout (Tr), salmon (Sa), frog (Fr), lizard (Lz), goose (Go), chicken (Ch), pigeon (Pi), turkey (Tu), donkey (Do), goat (Gt), horse (Ho), sheep (Sh), rabbit (Rab), calf (Ca), human (Hu) groups A, B, O and AB erythrocytes.

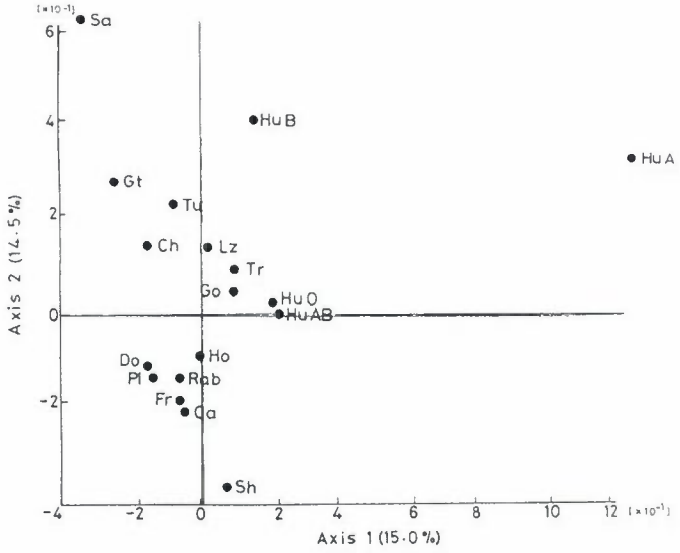

Figure 4. Corresponding ordination of erythrocyte types (•) based on haemagglutinin titres of the lichen species. (Full names of abbreviated erythrocyte types as per Fig. 3).

salmon, human A and B and to a lesser extent sheep reacted differently from the other erythrocyte types examined (Fig. 4). Donkey, horse, pigeon, rabbit, frog, and calf erythrocytes gave similar HGN levels as did the remaining 6 erythrocyte types which were also separately grouped together.

Correspondence analysis plots

The % of the total variation of the data explained by the diagram axes for HLY (Figs. 1 and 3) and HGN (Figs. 2 and 4) are 30.9% and 29.5% respectively. Although these percentages are low, this nevertheless is the best 2-dimensional representation of the data that could be obtained. In addition, interpretable patterns could be discerned.

Effect of habitat

Nine of the lichens used in the study were found both on rocky sea shores and in woodland adjacent to the shore. Furthermore all the lichen species obtained from each habitat gave positive reactions against chicken erythrocytes. Thus the mean and individual HGN and HLY titres were each compared between samples collected from the two different environments. The results are

given in Table 4 together with the levels of significance. Overall the HGN titres of the woodland samples were higher, and significantly different in five cases, than those of the sea shore whereas the converse in true for the HLY levels which showed significant differences in only two instances.

4. Discussion

The presence of relatively non-specific HGN and HLY and range of titres for maritime lichens is consistent with the findings for terrestrial lichen species (Barrett and Howe, 1968; Estola and Vartia, 1955). Furthermore, specificity for erythrocyte types of the human ABO(H) blood group has been reported for some crustose (Barrett and Howe, 1968; Fillho et al., 1980), foliose (Hardman et al., 1983) and fruticose lichens (Barrett and Howe, 1968). In the current study comparisons of mean and individual HGN or HLY titres, derived from bulk data of each of the morphological forms, for each of the erythrocyte types revealed non-significant differences ($P<0.05$) between foliose and fruticose against human AB (both HLY and HGN), and foliose and crustose lichens (HLY only) with human O and horse erythrocytes. In addition, all the lichen extracts gave similar degrees of agglutination albeit variable titres which seemed to depend upon the lichen species and erythrocyte types examined, irrespective of thallus form in many instances. It is possible that the use of selected erythrocyte types may reflect the naturally-occurring levels, spanning a wide range in titre from 2^{-1} to 2^{-9}, of HGN/HLY present in maritime lichen thalli. The low titres recorded for many of the extracts against several of the erythrocyte types are difficult to explain. They may simply be a consequence of either low levels of agglutinin synthesis, different extraction times or slight variations in lichen collection sites. To reduce protocol differences, batches of lichens were gathered from the same or similar sampling location, assays repeated and erythrocytes obtained from the same sources. Additionally, the possibility cannot be rules out that free, soluble sugars (formed as products of photobiont photosynthesis) released during the extraction procedure could inhibit haemagglutination. This might account for the finding of low titres and apparent absence of lectins in several of the extracts.

Of all the maritime species examined in the present work, crustose forms especially *L. atra* and *R. geographicum* displayed the most interspecies variation with respect to the amounts of both HLY and HGN. Furthermore, the largest intermorphological differences in HGN and HLY titres occurred between crustose and foliose lichens and the smallest between fruticose and foliose forms. Moreover the least inter- and intrageneric variations, and hence

closely related titres, were found with fruticose and certain foliose species respectively. In addition, the most intrageneric titre dissimilarities occurred in *Parmelia* and *Lecanora* species. The finding that some of the species, for example *B. chlorophaea* and *R. fastigiata*, behaved differently in their HGN and HLY reactivities compared to many of the other species tested is not easy to interpret. On the one hand, it is feasible that different chemical strains or races, or even chemically distinct sub-populations (Lawrey, 1984), of certain maritime lichen species of a given morphological form may vary from others of similar morphology in this respect. Alternatively, the broad intrageneric HGN titre variations detected in *Parmelia* and *Lecanora* may also be due to the above or be caused by a combination of environmental parameters, such as defined substrate or habitat specificity within the same area, which may affect metabolism and hence quantity of lytic and agglutinatory substances synthesized. The experimental data obtained in the current study indicates a wide variation in haemagglutination and haemolysis patterns. This suggests that some of the lichens may possess an HGN or HLY non-specifically reactive towards certain erythrocytes and/or different HGN (heteroagglutinins) and HLY molecules multispecific for various sugar residues on the surfaces of several erythrocyte types. In this context, Blunden and co-workers (1978) reported an unsuccessful attempt to classify marine algae on the basis of haemagglutination patterns. Whether taxonomic classification of lichens or indeed other lower plant species can be achieved using differences in HGN and/or HLY patterns remains tentative at the moment.

Some of the erythrocyte types employed in the present work (e.g. salmon, human A and B) are discrete from the majority of erythrocytes examined in their abilities to be agglutinated and lysed by the lichen extracts. This may be a result of variations in either carbohydrate moieties (receptors?) or stereochemical configurations of epitopes on the erythrocyte surface (Samuelsson and Breimer, 1987). It is known that the immunodominant terminal sugars on the membrane of human A and B erythrocytes are N-acetylgalactosamine and galactose respectively (Watkins, 1987) and it is likely that these sugars are recognised by a limited number of the lichen species tested. However, the chemical nature of the blood group specific determinants on the erythrocyte membrane of other vertebrates remains undetermined. Of the maritime lichen species used here, sugar-binding specificities have only been elucidated for the *C. pyxidata* anti-rabbit erythrocyte lectin (Ingram and Tassabehji, 1988b). In this instance HGN activity was inhibited by several carbohydrates (glucose, methyl glucoside, mannitol, mannosamine, N-acetylmannosamine, N-acetylglucosamine and N-acetylgalactosamine). By comparison, the lectins

from non-maritime forest lichens are specific for 6-deoxy-L-galactose and N-acetylglucosamine (Hardman et al., 1983) and galactose (Hardman et al., 1983; Petit, 1982). Therefore the heterogeneity of lichen agglutinins may enable a survival advantage by participation in the recognition and counteraction of the diverse array of potential environmental antigens. This tentative defence function is further supported by the fact that some lichen substances possess antibiotic properties which could possibly render protection and resistance of lichens to both saprobionts and pathogenic micro-organisms under natural conditions (Lawrey, 1986).

Some evidence has been provided that algal-binding proteins and lectins (agglutinins) in lichens, derived from the fungal symbiont, may mediate algal-fungal recognition, contact and/or attachment during thallus initiation and formation (Bubrick et al., 1985). In maritime lichen species examined in the present study, the mycobionts are ascomycetes which are known to produce lectins (Bouchara et al., 1987; Zeringue et al., 1982) and photobionts, with two exceptions, are exclusively *Trebouxia* spp. The exceptions are *L. incana* where the green algae involved are *Chlorella* and *Stichococcus*, and *Lecidea* spp. which possess *Chlorella, Myrmecia, Coccobotrys, Pleurococcus, Chlorosarcina* or *Trebouxia* (Duncan, 1970). However, there is no evidence to date to suggest that lichen blue-green or green algal symbionts synthesise and secrete lectins although *Trebouxia* and mycobionts isolated from lichens possess cell wall receptors for lectins of known sugar specificity (Marx and Peveling, 1983). Nevertheless the filamentous, freeliving, cyanophytan marine *Lyngbya majuscula* and terrestrial *Anabaena azollae* (the endosymbiont of the fern *Azolla*) produce anti-human erythrocyte agglutinins (Ingram, 1985a; Kobiler et al., 1982) and a unicellular, freeliving, chlorophytan freshwater alga *Chlamydomonas* synthesises sexual agglutinins, involved in cell-cell contact and adhesion, which are responsible for flagella-flagella pairing during the mating process (Tomson et al., 1990; Van DenEnde et al., 1986). In view of the above, it is feasible that fungi may secrete onto their surface or into the immediate surrounding lectins (agglutinins), with varying reactivities, which recognise an appropriate sugar receptor on the cell wall of their specific alga and hence cause binding and ultimately thallial synthesis. Further studies need to be undertaken into the biological roles of lichen lectins especially with regard to their potential involvement in anti-microbial defence mechanisms and thallus formation.

It remains speculative as to why maritime woodland lichens possess greater amounts of HGN and lower levels of HLY than the same sea shore species. It is possible that several environmental factors such as climate (including temperature fluctuations and amount of rainfall) and season, both of which

influence lichen growth (Lawrey, 1984), affect the physiology and metabolism of mycobiont-phytobiont associations and hence favour higher production of HGN in woodland and HLY in rocky sea shore lichens. Alternatively, the likelihood that variations in ecological habitat parameters may also cause differences in levels of these substances cannot be disregarded. Woodlands are more favourable habitats than sea shores and thus would favour the growth of harmful micro-organisms which, if one assumes a protective benefit, would explain the higher HGN levels. In work reported here, the amounts of HGN and HLY in extracts of several autumn collected lichen species were slightly higher than those found in summer samples against certain of the erythrocyte types. Furthermore, some of the previously negative findings for summer obtained lichens were positive in preparations of autumn collected samples. It has been shown that the HLY content of higher fungi fluctuates according to collection locations (Seeger et al., 1973) and season (Seeger and Wiedmann, 1972) and that the HGN levels in the organs of certain angiosperms seasonally vary. For example, bark lectins in elderberry (*Sambucus niger*) and black locust (*Robinia pseudoacacia*) are elevated in autumn and winter and decline in spring and summer (Nsimba-Lubaki and Peumans, 1986). Furthermore, lectins are found in various tissues during different stages of the winged bean *Psophocarpus tetragonolobus* life cycle and these molecules are assumed to be involved in the regulation of the growth process from germination upto the time of seed maturation (Shet and Madaiah, 1987). Moreover, in some higher plants agglutinins are deposited in bark and leaf protein-storage vacuoles (Herman et al., 1988) and in bark phloem parenchyma protein bodies (Greenwood et al., 1986). The latter authors postulated that the elderberry lectin may be either a storage compound or play a physiological role in storage and/or annual rhythmic mobilisation for plant development. However such lectins may not perform a storage function in lichens. Recently, Galun and co-workers (personal communication) have isolated a cyanolichen lectin, comprised of two approximately 50 kDa subunits, which appears to act as a mediator for the symbionts, a situation similar to that shown in *X. parietina* (Bubrick et al., 1985). Nevertheless, HGN (lectin) titres in maritime *C. pyxidata* samples are affected by exposure and aspect of sea shore, heavy metal and acid rain pollution, temperature, pH and sea-spray salt content (Ingram and Tassabehji, 1988a). It is apparent, from the findings of the current work, that the amounts of lichen HGN and HLY vary quantitatively according to time of year and site of collection, with very slight reduction in HGN and/or HLY activity due to low temperature storage. The environmental parameters involved in the regulation of biosynthesis of these substances

in maritime and indeed non-maritime lichens also warrant further investigation. Such work will hopefully elucidate the ecological functions of lichen HGN and HLY.

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