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Vertical Distribution of Plant Species with Binucleate or Trinucleate Pollen Influenced by Ultraviolet Radiation and Temperature

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Summary

The number of pollen nuclei was determined on 133 species distributed along the slope of mountain, and the number of species with trinucleate pollen had a tendency to increase at high altitudes more than at low altitudes.

From the relation between the number of pollen nuclei and the strength of the environmental stress, species with trinucleate pollen were statistically concluded to distribute under the higher stress of UV radiation or temperature than the case of species with binucleate pollen, and the distribution in trinucleate species was closely related to temperature more than UV radiation.

Various physiological and biochemical characters in trinucleate pollen, which have been suggested to be effective to avoid UV radiation stress, seem to be in the same situation also to temperature stress.

Introduction

Flowering plants in angiosperm shed binucleate or trinucleate pollens. It is cytologically known that binucleate pollen is shed after the mitosis of pollen cell while trinucleate pollen after the mitosis of a generative cell in the pollen. Many studies on the number of nuclei in these pollens have been taken from a physiological point of view.

BREWBAKER[2] has discussed a physiological significance in the trinucleate condition of pollen which is easily found in phylogenetical developed taxa, and has suggested an adaptive development of pollen from binucleate to trinucleate condition with environmental changes such as a change from humidic to xeric condition, the increase of UV radiation, etc.. In these environmental factors, UV radiation is well known to be a strong source for genetic varia-

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tion, and it has been reported that UV-B radiation damaged pollen at its various developmental stages[6,11]. On the other hand, trinucleate pollen has a possibility that provides a high resistance to UV-B rediation[6]. However, any analytical experiment on binucleate and trinucleate pollens has not been reported yet on temperature although the temperature is physiologically and ecologically one of important environmental factors to plants.

This paper will report the influence of UV radiation and temperature to vertical distribution of both species with binucleate and trinucleate pollens on the base of statistical analysis.

Materials and Methods

To investigate vertical distribution of plant species with binucleate or trinucleate pollen, an experimental area was set up on the eastern slope of Mt. Norikura (3026 m alt.) in central Japan. The lowest site of the slope was in about 600 m alt.

Pollens were collected from 133 species growing at various altitudes in this area during plant flowering season. Most of these species were forb, and a few woody plants were involved. Most grasses ware excepted. The collected pollens were stained by acetocarmine, and were observed to determine the number of pollen nuclei.

To analyze the relation between the number of pollen nuclei and the strength of environmental stress, the strengths of UV radiation and temperature stresses, which are quite influential to plant life, in living sites of plant species were relatively estimated for reproductive organs in opening flower in the field. As the strengths of both stresses are changed by some biological factors such as altitudinal plant habitat, plant life-habit, floral aspect during flowering time, and flowering season, the strengths were shown as the total of the partial stresses estimated individually to each of the factors. For the individual estimation, the stress on the altitudinal habitat was estimated by using altitudinal meteorological data, and the stress on the plant habit at the same altitude was estimated on the base of the relatively strength of solar radiation. The stress on the floral aspect was estimated from the facing position of opening flower. Stress estimation on flowering season was based on day length during the season for UV radiation stress and on average air tempererature during the season for temperature stress. Standard values to estimate the stresses of such various factors were given in Table 1.

For statistical treatment, binucleate and trinucleate species estimated on UV radiation or temperature stress were arranged in 10 classes from 0 to 9 according to those stress values. The relation between the number of pollen

Table 1 Standard values to relatively estimate the strength of environmental stress to reproductive organs in opening flower under various conditions of biological factors.

A. UV radiation

Biological factors	Conditions and standard values			
Altitudinal habitat	Basal zone	: 0	Montane zone	: 1
	Subalpine zone	: 2	Alpine zone	: 3
Life habit	In forest	: 0	Forest ridge	: 1
	Open field	: 2		
Flower aspect	Facing downward	: 0	Facing sideward	: 1
	Facing upward	: 2		
Flowering season	Mar. – Apl. Sep. – Oct.	: 0	May Aug.	: 1
	Jun. – Jul.	: 2		

B. Temperature

Biological factors	Conditions and standard values			
Altitudinal habitat	Basal zone	: 3	Montane zone	: 2
	Subalpine zone	: 1	Alpine zone	: 0
Life habit	In forest	: 0	Forest ridge	: 1
	Open field	: 2		
Flower aspect	Facing downward	: 0	Facing sideward	: 1
	Facing upward	: 2		
Flowering season	Mar. – Apl. Sep. – Oct.	: 0	May – mid. Jul. mid. – late Aug.	: 1
	mid. Jul. – mid. Aug.	: 2		

nuclei and the strength of UV radiation or temperature stress was investigated by Kolmogorov-Smirnov's two samples statistic test, and Cramér's association coefficient was calculated on UV radiation and temperature factors to compare an association degree between the distribution of trinucleate pollen species and the strength of an environmental stress.

Results

The plant species investigated, the number of pollen nuclei in these species, and the total stress values estimated on UV radianion and temperature were shown in Table 2. In most species, plants with binucleate or trinucleate pollen in a single family were found as a difference of genus, but *Plantago* in Plantaginaceae and *Viola* in Violaceae involved binucleate and trinucleate

Table 2 The number of pollen nuclei and the environmental stress values estimated on some plant species in Mt. Norikura.

Species	No. of nuclei	Stress value	
		UV radiation	Tempe-rature
Liliaceae			
Allium tuberosum	2	3	7
A. thumbergii	2	5	5
Erythronium japonicum	2	3	6
Gagea japonica	2	3	7
Hosta longissima	2	1	5
Lilium medeoloides	2	7	5
L. leichtlinii var. *maximowiczii*	2	5	5
Majanthemum dilatatum	2	5	3
Trillium smallii	2	5	6
Veratrum stamineum	2	6	5
Gramineae			
Bromus catharticus	3	5	8
Dactylis glomerata	3	6	9
Poa acroleuca	3	4	5
Iridaceae			
Iris sanguinea	2	2	3
Araceae			
Lysichiton camtshatcense	2	1	2
Symplocarpus foetidus var. *latissimus*	2	1	2
Orchidaceae			
Gymnadenia conopea	2	5	4
Orchis aristata	2	7	4
Commelinaceae			
Commelina communis	2	2	6
Scrophulariaceae			
Euphrasia maximowiczii	2	3	5
Mazus miquelli	2	3	4
Pedicularis chamissonis var. *japonica*	2	8	5
P. resupinata var. *oppositifolia*	2	6	5
P. yezoensis	2	7	5
Veronica nipponica	3	8	5
V. caninotesticulata	3	4	7
V. onoei	3	7	5
V. schmidtiana var. *senanensis*	3	7	5
Veronicastrum sibiricum	2	6	4
Primulaceae			
Lysimachia barystachys	2	5	8
L. clethroides	2	5	8
Primula cuneifolia var. *hakusanensis*	2	8	6

Trientalis europaea	2	6	4
Ericaceae			
Menziesia pentandra	2	4	3
Phyllodoce aleutica	2	5	3
Rhododendron japonicum	2	5	6
R. aureum	2	7	4
R. Tschonoskii	2	6	6
Vaccinium Vitis-Idaea	2	7	4
Dispensiaceae			
Shortia soldanelloides	2	7	5
Gentianaceae			
Gentiana zollingeri	3	4	7
Halenia corniculata	3	5	6
Adoxaceae			
Adox moschatellina	3	3	5
Valerianaceae			
Patrinia triloba var. *triloba*	3	7	7
Dipsacaceae			
Scabiosa japonica	3	6	5
Compositae			
Arnica unalaschcensis var. *Tschonoskyi*	3	9	6
Achillea sibirica	3	8	6
Erigeron philadelphicus	3	7	5
Ixeris stolonifera	3	5	8
Ligularia fischeri	3	6	7
Leontopodium japonicum	3	8	4
Picris hieracioides ssp. *japonica*	3	8	7
Solidago virgaurea ssp. *asiatica*	3	6	7
Taraxacum hondoense	3	7	7
Labiatae			
Ajuga nipponensis	2	3	6
Clinopodium chinense var. *parviflorum*	3	6	7
Elsholtzia ciliata	3	5	7
Lamium purpureum	2	4	7
Prunella vulgaris ssp. *asiatica*	3	6	7
Campanulaceae			
Adenophora triphylla var. *japonica*	2	2	5
A. remotiflora	2	2	3
A. nikoensis	2	5	5
Asyneuma japonicum	2	3	4
Campanula lasiocarpa	3 (2)	8	4
C. punctata	2	3	3
Caprifoliaceae			
Viburnum furcatum var. *radiata*	3	8	7
Plantaginaceae			
Plantago asiatica	2	5	7

P. lanceolata	3	5	7
Solanaceae			
Scopolia japonica	2	1	3
Rosaceae			
Agrimonia pilosa var. *japonica*	2	3	4
Duchesnea indica	2	3	6
Fragaria nipponica	2	7	5
Geum pentapetalum	2	9	6
Kerria japonica	2	4	5
Potentilla fragarioides var. *maior*	2	5	7
P. Freyniana	2	5	7
P. Dickinsii	2	8	7
Legminosae			
Astragalus sinicus	2	3	6
Hedysarum vicioides	2	7	4
Trifolium Lupinaster	2	6	4
T. repens	2	4	6
T. pratense	2	4	6
Vicia villosa	2	3	3
V. angustifolia var. *segetalis*	2	3	6
V. Cracca	2	4	6
Caryophyllaceae			
Cerastium pauiflorum var. *oxalidiflorum*	2	6	6
Dianthus superbus var. *speciosus*	3	9	6
Lychnis Miqueliana	3	5	7
Moehringia lateriflora	3	5	6
Pseudostellaria Palibiniana	3	6	7
Ranunculaceae			
Anemone flaccida	2	3	5
A. Raddeana	2	4	4
A. narcissiflora	2	9	6
Aquilegia Buergeriana	2	4	5
Clematis stans	2	3	4
Coptis trifolia	2	6	5
Glaucidium palmatum	2	7	5
Isopyrum raddeanum	2	1	3
Ranunculus acris var. *nipponicus*	2	7	6
R. japonicus	2	5	7
Thalictrum aquilegifolium	2	5	6
T. filamentosum var. *tenerum*	2	5	3
Trautvetteria japonica	2	5	6
Trollius Riederianus var. *japonicus*	2	8	5
Geraniaceae			
Geranium eriostemon var. *reinii*	3	8	7
G. yesoense var. *nipponicum*	3	9	7
Crassulaceae			

Sedum erythrostictum	2	6	6
S. aizoon	2	6	4
Oxalidaceae			
Oxalis corniculata	3	6	9
Guttiferae			
Hypericum erectum	2	5	6
H. kamtschaticum var. *senanense*	2	7	5
Cruciferae			
Cardamine flexuosa	2	2	4
Violaceae			
Viola mandshurica	2	4	5
V. biflora	3	8	5
V. vaginata	2	3	4
Saxifragaceae			
Chrysosplenium macrostemon var. *shiobarense*	2	3	4
C. ramosum	2	4	4
Parnassia palustris	3	5	8
Saxifraga fortunei var. *incisolobata*	2	4	4
S. fusca var. *kikubuki*	2	6	5
Aceraceae			
Acer ukurunduense	2	6	5
Umbelliferae			
Bupleurum longiradiatum for. *elatius*	3	7	6
Berberidaceae			
Caulophyllum robustum	2	4	4
Diphylleia Grayi	2	6	4
Epimedium grandiflorum ssp. *koreanum*	2	3	4
E. grandiflorum	2	3	4
Ranzania japonica	2	5	3
Aristolochiaceae			
Asarum sieboldii	2	3	2
Balsaminaceae			
Impatiens Textori	2	2	6
Cornaceae			
Chamaepericlymenum canadense	2	5	5
Onagraceae			
Oenothera erythrosepala	2	3	7
Papaveraceae			
Corydalis heterocarpa var. *japonica*	2	2	4
C. incisa	2	2	4

species. As a special species, *Campanula laciocarpa* among alpine plants was found to release the mixed pollen grains of bi- and tri-nucleus from a single flower, and trinucleate pollen ratio to the total number of pollens ranged from about 30% to about 50%.

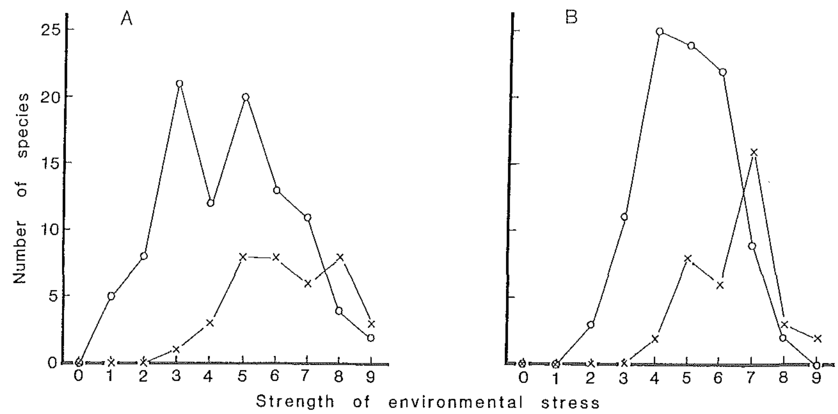

Fig. 1 The mumber of species with binucleate pollen (○) or trinucleate pollen (×) varied by relatively strengths of UV radiation (A) and temperature (B) stresses.

Out of 133 species investigated, 96 species were with binucleate pollen, and 37 species had trinucleate one. Species which originated from higher altitudes had a tendency to produce trinucleate pollen in a higher rate than species from lower altitudes.

The relation between the number of species with binucleate or trinucleate pollen and the stress strengths was shown in Fig. 1. *Campanula laciocarpa* was classified as trinucleate. In the relation, the maximum number of species with trinucleate pollen was found at the higher levels of UV radiation and temperature stresses than the case of species with binucleate pollen, and was found in higher stress of temperature than that of UV radiation. The variation in the number of trinucleate species along with the increase of these stresses was characterized by the maximum at a few classes of the stress on UV radiation while at its one class on temperature.

A difference in the tendency described above between both species with binucelate and trinucleate pollen was statistically significant with 1% error to Kolmogorov-Smirnov's two samples statistic test on both factors of UV radiation and temperature, and species with trinucleate pollen were concluded to distribute under the higher stress of UV radiation or temperature than the case of species with binucleate pollen. Cramér's association coefficient between the distribution of trinucleate species and stress strengths was 0.185 on UV radiation factor and 0.268 on temperature's. This coefficient is defined as the perfect association when the value is 1. Accordingly, it resulted that the distribution of trinucleate species into the habitat showing high environmental stress was closely related with the temperature rather than with the UV radiation factor.

Discussion

As plants protect themselves from environmental stress by various means, practical injurious effects of the environmental stress to them must be considered in relation to their protective ability. The UV radiation injury in pollens seems to arise during the time from pollen dispersal to just before pollen tube penetration into stigmatic tissue because of high UV radiation screening ability of anther wall[5] and stigma[12]. On the other hand, temperature will influence various physiological activities during the time from pollen germination to fertilization. For this reason, the influence of environmental stress to pollen may be larger in the case of temperature factor than UV radiation one. A difference in the strength of environmental stress arises also from different flowering times in a day. The flowering time, however, was not considered at present because, in all the species investigated, only an exceptional species was *Oenothera erythrosepara*, a noctural one.

The increase of the UV radiation stress means that the injurious effect of UV radiation is gradually increased. The increase of the temperature stress, however, is different from the case of UV radiation. On the temperature stress, its medium level is accelerative and never inhibitory to physiological processes in many kinds of plants. Furthermore, although air temperature is largely lower in alpine zone than in basal one, the temperature stress to alpine flowers, especially heliotrophic one, seems to show such high value as flowers in basal zone because high solar radiation at high altitudes may easily heighten the temperature of materials radiated. The temperature heightened by the solar radiation, however, rapidly lowers after the sun is hidden by clouds. Therefore, the temperature factor in high altitudes must be considered with such temperature conditions, and it means that the temperature stress estimated on the highest temperature in its drastic fluctuation is never represented by only the high level of temperature because, especially, the high temperature in alpine climate is certainly accompanied by a inhibitory low temperature on many species.

A fact on the environmental stress value estimated here is that individual stresses in the same value among different biological factors are probably different to each other on the practical strength of the stress. At present, however, the total of such individual stress values was used to estimate the strength of the stress to which species is exposed. This procedure seems to be better to generalize the stress strength in various conditions.

On the number of pollen nuclei, BREWBAKER[2] has reported that each of binucleate and trinucleate pollens is rarely found on different species in a genus and never in the same species. At present, however, it was found that *Campanula lasiocarpa* shed the mixed pollens of binucleus and trinucleus from a single flower. The family Campanulaceae has been reported to consist of binucleate and trinucleate pollen genera[2], yet species with the mixed pollens had never been known. Whether the mixed pollens on the number of nuclei relate with anther age[15] or suggest a process of the evolutional differentiation of pollen from binucleate to trinucleate is not clear now, although the present pollens were collected from mature anthers which were actively releasing the pollens.

In the relation between the number of species and the strength of environmental stress, binucleate species distributed bimodually to the UV radiation stresses, but it is not clear at present whether the distribution pattern is essential or not. Although binucleate species make such an indistinct distribution to UV radiation stress, the statistical result that species with trinucleate pollen distribute in higher UV radiation stress than species with binucleate one supports FLINT and CALDWELL's suggestion[6] that trinucleate pollen may provide a higher resistance to UV-B radiation than binucleate pollen.

Further findings are that the trinucleate species distribution into higher environmental stress than the case of binucleate species is statistically significant not only on UV radiation but on temperature also, and that trinucleate species distribution in a habitat is likely to be related with the temperature factor more than with the UV radiation one. Although any evidence to verify the influence in temperature is not yet obtained, some results suggested to avoid UV radiation injury in trinucleate pollen, such as more quick germination[1,8], larger growth rate of pollen tube[3,7], and a shorter interval from pollination to fertilization[4,9,13] in trinucleate pollen than binucleate one, may be very useful also to avoid disadvantageous temperature stresses. Practically, UV radiation effect to pollen tube growth seems to be very low or negligible because pollen tube growth is mostly related to the inners of styl which has a UV radiation screening effect[14], but temperature effect during its growth is never negligible. At high altitudes rather than at low ones, styl's temperature may be easily brought to physiologically its unsuitable level, low or high one, through a frequnt change of direct solar radiation. KEVAN[10] has reported a large fluctuation of intrafloral temperature within a short time in high arctic flowers. In our preliminary experiment, the intrafloral temperature in opening flowers of some alpine plants was about 5°C higher than an ambient temperature in sunny condition, but decreased to about the ambient level within 1 min. after shading flowers. Under such temperature conditions, the srecific characteristics of trinucleate pollen described above would be very useful to carry out from pollination to fertilization within a short time during which an optimum temperature is being kept.

More species with trinucleate pollen found at high altitudes than at low ones seem to result from their high ability to avoid a disadvantage which would be caused by UV radiation and temperature stresses. In this case, the ability may be related to temperature more than UV radiation. These results suggest that the establishment of new species into high altitudes is easier on species with trinucleate pollen than on species with binucleate one and is limited by UV radiation more than by temperature.

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