



## OPEN

## SUBJECT AREAS:

EVOLUTIONARY  
ECOLOGY

PLANT EVOLUTION

Received  
24 October 2013Accepted  
6 March 2014Published  
31 March 2014Correspondence and  
requests for materials  
should be addressed to  
Y.-W.D. (duanyw@  
mail.kib.ac.cn)

# Pollen sensitivity to ultraviolet-B (UV-B) suggests floral structure evolution in alpine plants

Chan Zhang<sup>1,2,3</sup>, Yong-Ping Yang<sup>1,2,4</sup> & Yuan-Wen Duan<sup>1,2,4</sup>

<sup>1</sup>Key Laboratory for Plant Biodiversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China, <sup>2</sup>Institute of Tibetan Plateau Research at Kunming, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China, <sup>3</sup>Graduate University of the Chinese Academy of Sciences, Beijing 100049, P. R. China, <sup>4</sup>Plant Germplasm and Genomics Center, the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204, P. R. China.

Various biotic and abiotic factors are known to exert selection pressures on floral traits, but the influence of ultraviolet-B (UV-B) light on the evolution of flower structure remains relatively unexplored. We have examined the effectiveness of flower structure in blocking radiation and the effects of UV-B on pollen viability in 42 species of alpine plants in the Hengduan Mountains, China. Floral forms were categorized as either protecting or exposing pollen grains to UV-B. The floral materials of plants with exposed and protected pollen grains were able to block UV-B at similar levels. Exposure to UV-B radiation *in vitro* resulted in a significantly greater loss of viability in pollen from plant species with protective floral structures. The pronounced sensitivity of protected pollen to UV-B radiation was associated with the type of flower structure. These findings demonstrate that UV-B plays an important role in the evolution of protective floral forms in alpine plants.

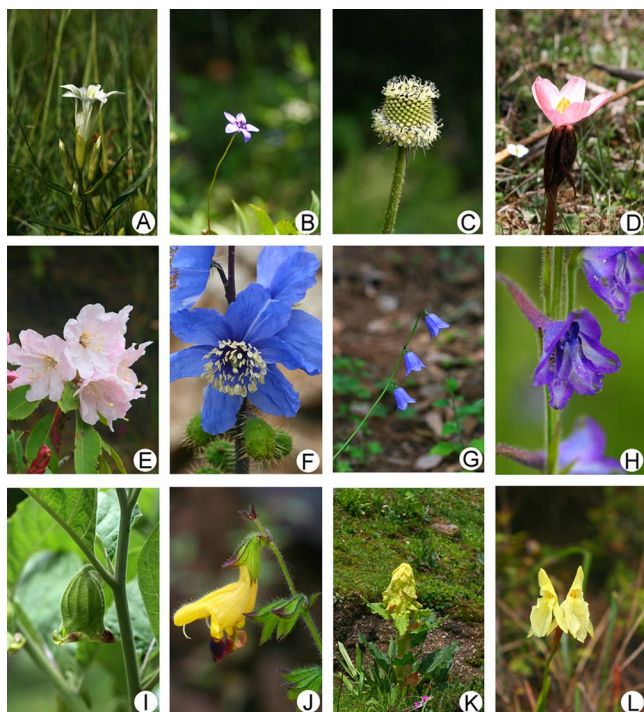
The evolution and diversification of floral traits are driven by both biotic and abiotic agents<sup>1–3</sup>. Historically, morphological differences in floral traits have been attributed to pollinator attraction and manipulation<sup>4,5</sup>. However, such factors as herbivory and environmental conditions may also play a strong role<sup>6,7</sup>. For example, anthocyanin-based polymorphic petal color may increase fitness during drought or periods of high heat<sup>1,8</sup>. Among the most well-studied agents is rain, which can wash away pollen grains, reduce pollen viability and dilute nectar<sup>9–12</sup>. Plant species that do not produce water-repellent pollen grains commonly exhibit floral traits that protect against pollen degradation due to wetting, suggesting that rain is an important selective force in shaping floral structure<sup>10,13–15</sup>. Ultraviolet-B (UV-B) is known to reduce pollen viability<sup>16–19</sup>, and strong UV-B radiation is a typical characteristic of alpine environments; however, the relationship between pollen sensitivity and protective floral forms remains largely unexplored<sup>13</sup>.

Although rain protection is likely a key driver of floral forms, it may not fully explain the floral evolution of alpine plants for two reasons. First, traits such as flower closure induced by temperature decline (seen in alpine gentians) protect pollen from rain but not from UV-B<sup>9,20</sup>. Second, traits such as pendulous flowers may be under simultaneous selection by both rain and UV-B<sup>21,22</sup>. In this study, we seek to clarify the selective pressure of UV-B on the floral traits of alpine plants using *in vitro* pollen germination experiments. We hypothesized that unprotected pollen grains located on UV-B exposed anthers (Fig. 1 A–F) would be less sensitive than pollen grains protected by flower structures such as bracts or petals (Fig. 1 G–L). We addressed the following specific questions: 1) What is the ability of flower structures to reduce UV-B exposure? 2) What is the difference in viability of protected and unprotected pollen after exposure to UV-B radiation?

## Results

More than 70% ( $74.0 \pm 2.5\%$ , mean  $\pm$  S.E.) of UV-B radiation was excluded by flower structures. The blocking ability of the material composing protective or potentially protective structures on both types of flowers was similar (Fig. 2A).

Pollen viability, measured by pollen germination, was similar in the control and UV-B treated pollen in 15 of the 42 plant species studied. In the remaining 27 species, pollen viability was reduced significantly after exposure to UV-B light (Table S1). Among these species, pollen from flowers with protective structures experienced a



**Figure 1** | Flower structures defined as exposing (A–F) and protecting (G–L) the pollen of several representative plant species from the high altitudes of the Qinghai-Tibet Plateau and adjacent areas (Photographs by Y.W.D.). (A). *Gentiana straminea*; (B). *Codonopsis convolvulacea*; (C). *Dipsacus asperoides*; (D). *Sinopodophyllum hexandrum*; (E). *Rhododendron decorum*; (F). *Meconopsis racemosa*; (G). *Adenophora khasiana*; (H). *Delphinium delavayi*; (I). *Anisodus luridus*; (J). *Salvia digitaloides*; (K). *Rheum alexandrae*; (L). *Roscoea cauleoides*.

**Table 1** | The association between floral protective structure and pollen sensitivity to UV-B

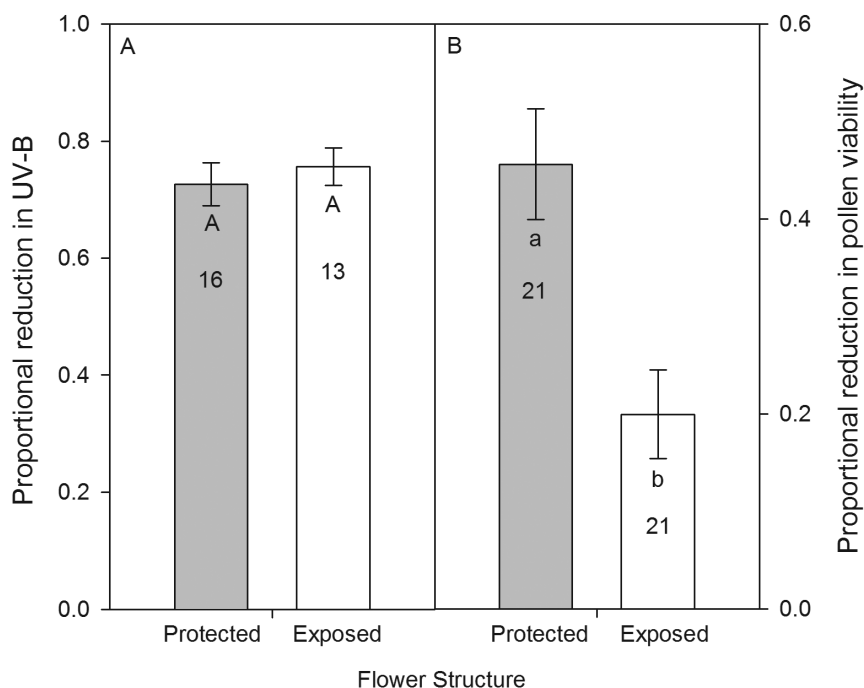
	Exposed	Protected
Insensitive	13	4
Sensitive	8	17

nearly 50% reduction in viability relative to the control, whereas pollen from exposed flowers exhibited only a 20% reduction (Fig. 2B). In general, pollen viability decreased more in pollen from flowers with protective structures, and the reduction in viability between protected and exposed pollen was significant (Fig. 2B). We also found a significant positive relationship between more protective flower structures and greater pollen sensitivity to UV-B ( $\chi^2 = 8.01$ ,  $P = 0.011$ ; Table 1).

## Discussion

The UV-B levels on the Qinghai-Tibet Plateau and adjacent highlands are much higher than in many low-altitude places, and they are at their highest during the flowering season (May–August)<sup>23,24</sup>. Thus, UV-B has the potential to inflict considerable damage on these reproductive structures, resulting in decreased pollen viability and lowered fitness of alpine plants<sup>16–19,25</sup>. Most of the alpine plants we tested contained pollen that was sensitive to UV-B, and pollen that was more sensitive to UV-B was also more likely to belong to species that featured protective floral structures. This observation suggests that UV-B has played a role in shaping the flower structure of alpine plants in the Qinghai-Tibet Plateau and adjacent highlands.

Three caveats should be considered. First, flower structures may protect pollen that is sensitive to UV-B through shading, but it remains unclear how exposed pollen grains protect themselves from damage caused by UV-B. It is possible that these pollen types have an increased pollen wall thickness<sup>26</sup> or contain isozymes or antioxidants<sup>27</sup>. Future research should investigate the mechanisms responsible for UV-B protection in these pollen grains. Second, pollen



**Figure 2** | The effects of UV-B on *in vitro* pollen germination. (A) Proportional reduction in UV-B by floral structures relative to control. (B) Proportional reduction in pollen viability after exposure to UV-B relative to control. The letters indicate that the difference was significant at the  $P = 0.01$  level, and the error bars indicate S.E. The number in each bar is the species sample size.



sensitivity to UV-B might be different in plant species that utilize different pollination systems. Our study only considered pollen from entomophilous plants, which we expect to be more sensitive to UV-B than pollen from anemophilous plants, such as the wind-pollinated *Aconitum gymnanthum*<sup>28</sup>, because insect-pollinated species are less exposed. Third, strong UV-B may affect pollen tube growth even after the grains germinate, but our *in vitro* experiments were limited to quantifying the effect of UV-B on pollen germination<sup>16,17</sup>. Although the wall of the style and the receptacle tissue surrounding the ovary are likely to form protective barriers against UV-B radiation, this type of protection has not been rigorously tested in alpine plant communities<sup>29</sup>.

Our work provides compelling evidence that flower structures can protect sensitive pollen from UV-B damage, indicating that UV-B radiation may play an important role in influencing the evolution of floral traits. Adaptable morphology in response to UV-B radiation may become increasingly relevant for these alpine communities as ozone levels decrease and more UV-B reaches the surface of the earth<sup>30</sup>. The impact of such global changes on floral evolution and/or plant distribution may be particularly evident in extreme alpine environments such as the Qinghai-Tibet Plateau.

## Methods

**Study species.** Our experiments were conducted from June to September 2011 in the Lijiang Forest Ecosystem Research Station (27°00' N, 100°10' E, 3,250 m) south of Jade Snow Mountain, Yunnan Province, China. Most of the plant species in this location are native, but some have been introduced from other areas of Yunnan Province. We only included native or introduced plants from high-altitude sites that represent alpine communities.

**Effectiveness of flower structures in preventing UV-B.** We divided plant species into two categories: plants with floral structures such as bracts or petals that could protect pollen from UV-B exposure and those that lacked such protective structures (Fig. 1). We then measured the effectiveness of flower structures in blocking UV-B.

For protective flowers, to test the proportion of UV-B excluded by flower structures, we used a digital portable UV radiometer (UV-B type, Photoelectric Instrument Factory of Beijing Normal University, Beijing, P. R. China) to examine the intensity of UV-B under and outside the flower parts (e.g., bracts, petals, sepals) that sheltered the anther from UV-B. For exposed flowers, we used the level of UV-B measured near the flowers as the exposure level of UV-B and placed the receptive area of the UV-B meter under the flower parts corresponding to the sheltering structures in the protective flowers to measure the proportion of UV-B that could be blocked. For plant species with large flowers, we measured the UV-B intensity *in situ*; for small flowers, we removed the flower structure from the plant and placed it immediately on the receptive area of the meter.

In total, we examined 29 plant species, 16 of which we classified as protective of the pollen grains (Table S1). We quantified the effectiveness of flower structures in preventing UV-B using the formula  $1 - N_u/N_o$ , where  $N_u$  and  $N_o$  were the intensity of UV-B under and outside the flower structures, respectively. The difference in the effectiveness of flower structures that protect and structures that expose pollen was analyzed using independent-sample Student's t-tests after performing a power transformation using the Box-Cox method.

**Effects of UV-B on pollen viability.** We used *in vitro* pollen germination to determine pollen viability. The pollen grains were harvested from newly opened anthers from at least 10 plants of each species; we used pollen from 42 plant species in 20 families (Table S1).

The anthers were placed in a centrifuge tube with 10 ml of 10% sucrose solution. After being thoroughly shaken to dislodge the pollen grains, the anther tissue was removed. The remaining solution was divided equally between two 9-cm-diameter Petri plates, each with a solid medium containing 15% sucrose, 1.5% bacto-agar, 0.01% H<sub>3</sub>BO<sub>3</sub>, 0.03% Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 0.02% MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.01% KNO<sub>3</sub> and 0.01% KH<sub>2</sub>PO<sub>4</sub><sup>31</sup>. One plate was designated the control, and the other plate was exposed to UV-B light with an intensity of 100 μW/cm<sup>2</sup>, similar to the intensity of UV-B during one hour of exposure to natural sunlight at the station. All plates were placed in an incubator at 25°C with visible light for eight hours. Upon removal, germination was halted on all plates by adding several drops of killing and preserving solution. This solution consisted of water, glycerine, formaldehyde and glacial acetic acid at a ratio of 72:20:5:3 by volume.

Pollen germination was determined using a light microscope at 100× magnification. At least 1000 pollen grains were scored for each plate. The germination rate for each plant species was analyzed using independent-sample Student's t-tests after power-transformation using the Box-Cox method. The pollen sensitivity was quantified as the proportional reduction in pollen viability relative to the control after exposure to UV-B using the formula  $1 - N_u/N_o$ , where  $N_u$  and  $N_o$  were the germination rates of the pollen grains exposed to UV-B and of the control grains,

respectively. For each species, pollen was classified as sensitive if the *in vitro* germination was significantly reduced relative to the control. The association between flower structures and pollen sensitivity was tested by chi-square in a cross table.

1. Strauss, S. Y. & Whittall, J. B. Non-pollinator agents of selection on floral traits. In Harder, L. D. & Barrett, S. C. H. (eds) *Ecology and Evolution of Flowers* (Oxford University Press, 2006).
2. Harder, L. D. & Johnson, S. D. Darwin's beautiful contrivances: Evolutionary and functional evidence for floral adaptation. *New Phytol.* **183**, 530–545 (2009).
3. Galen, C. & Cuba, J. Down the tube: Pollinators, predators, and the evolution of flower shape in the alpine skypilot, *polemonium viscosum*. *Evolution* **55**, 1963–1971 (2001).
4. Darwin, C. *On the Origin of Species by Means of Natural Selection or the Preservation of Favoured Races in the Struggle for Life*. (John Murray, 1859).
5. Fenster, C. B., Armbruster, W. S., Wilson, P., Dudash, M. R. & Thomson, J. D. Pollination syndromes and floral specialization. *Ann. Rev. Ecol. Evol. Syst.* **35**, 375–403 (2004).
6. Herrera, C. M. Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecol. Monogr.* **63**, 398–398 (1993).
7. Armbruster, W. S. Evolution of floral morphology and function: an integrative approach to adaptation, constraint, and compromise in *Dalechampia* (Euphobiaceae). In Lloyd, D. G. & Barrett, S. C. H. (eds) *Floral Biology* (Chapman & Hall, 1996).
8. Steyn, W. J., Wand, S. J. E., Holcroft, D. M. & Jacobs, G. Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. *New Phytol.* **155**, 349–361 (2002).
9. He, Y. P., Duan, Y. W., Liu, J. Q. & Smith, W. K. Floral closure in response to temperature and pollination in *Gentiana straminea* Maxim. (Gentianaceae), an alpine perennial in the qinghai-tibetan plateau. *Plant Syst. Evol.* **256**, 17–33 (2006).
10. Sun, J.-F., Gong, Y.-B., Renner, S. S. & Huang, S.-Q. Multifunctional bracts in the dove tree *Davidia involucreata* (Nyssaceae: Cornales): Rain protection and pollinator attraction. *Am. Nat.* **171**, 119–124 (2008).
11. Dafni, A. Autumnal and winter pollination adaptations under mediterranean conditions. *Bocconea* **5**, 171–181 (1996).
12. Huang, S.-Q., Takahashi, Y. & Dafni, A. Why does the flower stalk of *Pulsatilla cernua* (Ranunculaceae) bend during anthesis? *Am. J. Bot.* **89**, 1599–1603 (2002).
13. Mao, Y.-Y. & Huang, S.-Q. Pollen resistance to water in 80 angiosperm species: Flower structures protect rain-susceptible pollen. *New Phytol.* **183**, 892–899 (2009).
14. Aizen, M. A. Down-facing flowers, hummingbirds and rain. *Taxon* **52**, 675–680 (2003).
15. Galen, C., Edward, G. R. & Fakhri, A. B. It never rains but then it pours: the diverse of water on flower integrity and function. In Reekie, E. G. & Bazzaz, F. A. (eds) *Reproductive Allocation in Plants* (Elsevier Academic Press, 2005).
16. Torabinejad, J., Caldwell, M. M., Flint, S. D. & Durham, S. Susceptibility of pollen to UV-B radiation: An assay of 34 taxa. *Am. J. Bot.* **85**, 360–369 (1998).
17. Feng, H., An, L., Tan, L., Hou, Z. & Wang, X. Effect of enhanced ultraviolet-B radiation on pollen germination and tube growth of 19 taxa *in vitro*. *Environ. Exp. Bot.* **43**, 45–53 (2000).
18. van de Staaij, J. W. M., Bolink, E., Rozema, J. & Ernst, W. H. O. The impact of elevated UV-B (280–320 nm) radiation levels on the reproduction biology of a highland and a lowland population of *Silene vulgaris*. *Plant Ecol.* **128**, 173–179 (1997).
19. Demchik, S. M. & Day, T. A. Effect of enhanced UV-B radiation on pollen quantity, quality, and seed yield in *Brassica rapa* (Brassicaceae). *Am. J. Bot.* **83**, 573–579 (1996).
20. Bynum, M. R. & Smith, W. K. Floral movements in response to thunderstorms improve reproductive effort in the alpine species *Gentiana algida* (Gentianaceae). *Am. J. Bot.* **88**, 1088–1095 (2001).
21. Wang, Y., Meng, L.-H., Yang, Y.-P. & Duan, Y.-W. Change in floral orientation in *Anisodus luridus* (Solanaceae) protects pollen grains and facilitates development of fertilized ovules. *Am. J. Bot.* **97**, 1618–1624 (2010).
22. Song, B. *et al.* Multifunctional bracts enhance plant fitness during flowering and seed development in *Rheum nobile* (Polygonaceae), a giant herb endemic to the high Himalayas. *Oecologia*. *Oecologia* **172**, 359–370 (2013).
23. Rao, X.-Q. *Observation Study and Model Simulation of Solar Ultraviolet Radiation over the Qinghai-Xizang Plateau*. (Chinese Academy of Meteorological Sciences, Master Dissertation, 2003).
24. Zhu, Q. *et al.* Spatialization research on ultraviolet radiation in China. *Resour. Sci.* **27**, 108–113 (2005).
25. Flint, S. D. & Caldwell, M. M. Partial inhibition of *in vitro* pollen germination by simulated solar ultraviolet-B radiation. *Ecology* **65**, 792–795 (1984).
26. Yeloff, D., Blokker, P., Boelen, P. & Rozema, J. Is pollen morphology of *Salix polaris* affected by enhanced UV-B irradiation? Results from a field experiment in high arctic tundra. *Arct. Antarct. Alp. Res.* **40**, 770–774 (2008).
27. Wang, S. W. *et al.* Increased uv-b radiation affects the viability, reactive oxygen species accumulation and antioxidant enzyme activities in maize (*Zea mays* L.) pollen. *Photochem. Photobiol.* **86**, 110–116 (2010).



28. Duan, Y. W., Zhang, T. F., He, Y. P. & Liu, J. Q. Insect and wind pollination of an alpine biennial *Aconitum gymnandrum* (Ranunculaceae). *Plant Biol.* **11**, 796–802 (2009).
29. Day, T. A. & Demchik, S. M. Ultraviolet-B radiation screening effectiveness of reproductive organs in *Hesperis matronalis*. *Environ. Exp. Bot.* **36**, 447–454 (1996).
30. Andradý, A. *et al.* Environmental effects of ozone depletion and its interactions with climate change: Progress report, 2009. *Photochem. Photobiol. Sci.* **9**, 275–294 (2010).
31. Brewbaker, J. L. & Kwack, B. H. The essential role of calcium ion in pollen germination and pollen tube growth. *Am. J. Bot.* **50**, 859–865 (1963).

## Acknowledgments

We are grateful to Dr. Zhi-Kun Wu and Mr. Kun Xu in Lijiang Forest Ecosystem Research Station for their helps in identifying plant species and the logistical supports in the field experiments, and Prof. Moshe Inbar for his suggestions and efforts in polishing English to the early version of the manuscript. This work was financially supported by 973 programs (2010CB951704) and NSFC (31270434).

## Author contributions

Y.W.D. and Y.P.Y. designed the research and wrote the manuscript; Y.W.D. and C.Z. performed experiments; Y.W.D. analysed data and prepared the figures and tables.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Zhang, C., Yang, Y.-P. & Duan, Y.-W. Pollen sensitivity to ultraviolet-B (UV-B) suggests floral structure evolution in alpine plants. *Sci. Rep.* **4**, 4520; DOI:10.1038/srep04520 (2014).



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 3.0 Unported License. The images in this article are included in the article's Creative Commons license, unless indicated otherwise in the image credit; if the image is not included under the Creative Commons license, users will need to obtain permission from the license holder in order to reproduce the image. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>