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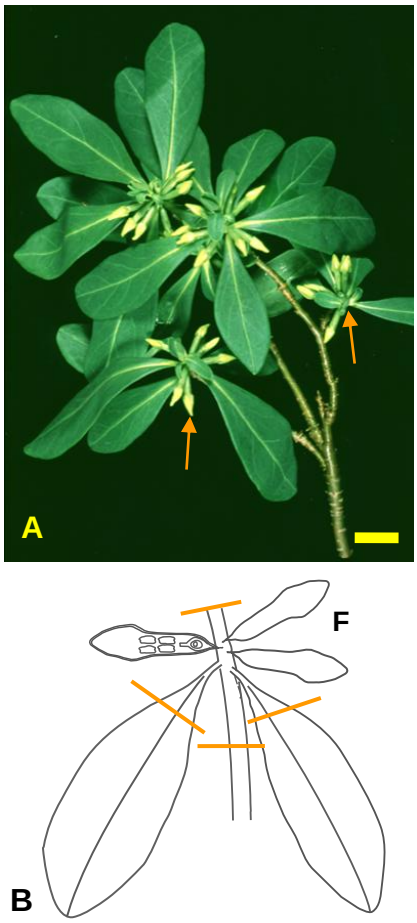

Fig. 1

Figure 1. A wintering hermaphroditic plant of *Daphne kamtschatica* var. *jezoensis* (A). A cluster of florets are directly attached to the internode adjacent to mature leaves (B). Stem segments with several florets were excised with their leaves trimmed as typically indicated by the orange bars in (B) and used for DTA and MRI imaging. The outer views of hermaphroditic and female florets are similar except that the former tends to be slightly larger and more yellowish than female florets (Fig. 8). F, florets; LB, Leaf buds. The bar indicates 10 mm.

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Fig. 2

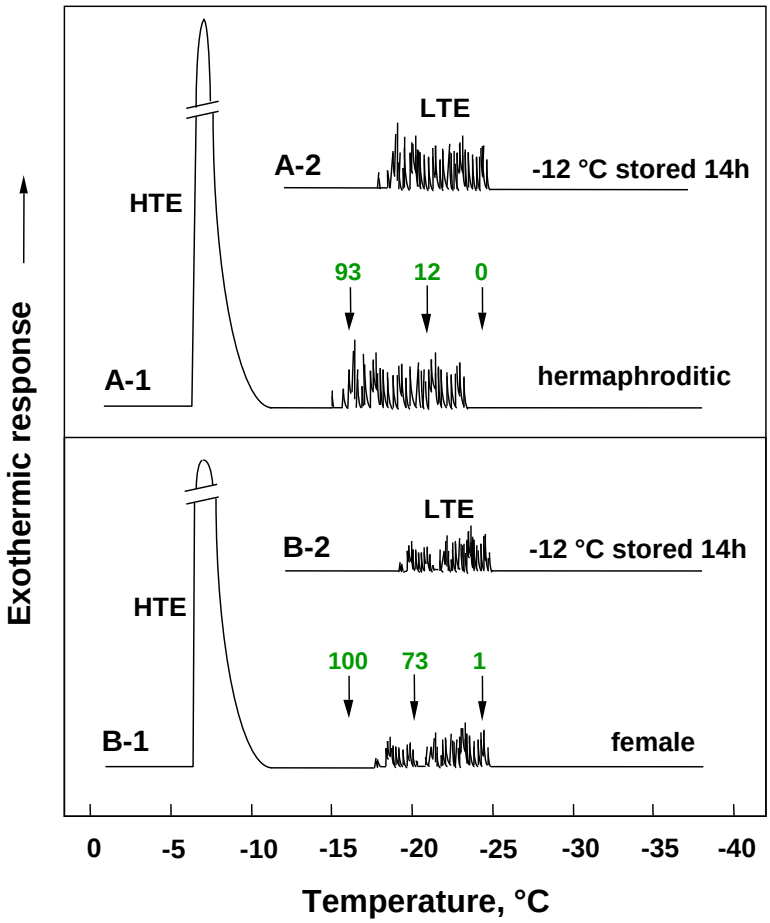

Figure 2. Typical DTA profiles of hermaphroditic (A) and female (B) *Daphne* flower buds collected in January. In DTA profiles A-1 and B-1, the buds were cooled at 5 °C/h from 0 to -30 °C. In profiles A-2 and B-2, the buds were maintained at -12 °C for 14 h before being further cooled at 5 °C/h. The numbers above the numerous spike-like LTEs are the % of live anthers when the plants were taken out of the freezer at the temperatures indicated by the arrows.

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Fig. 3

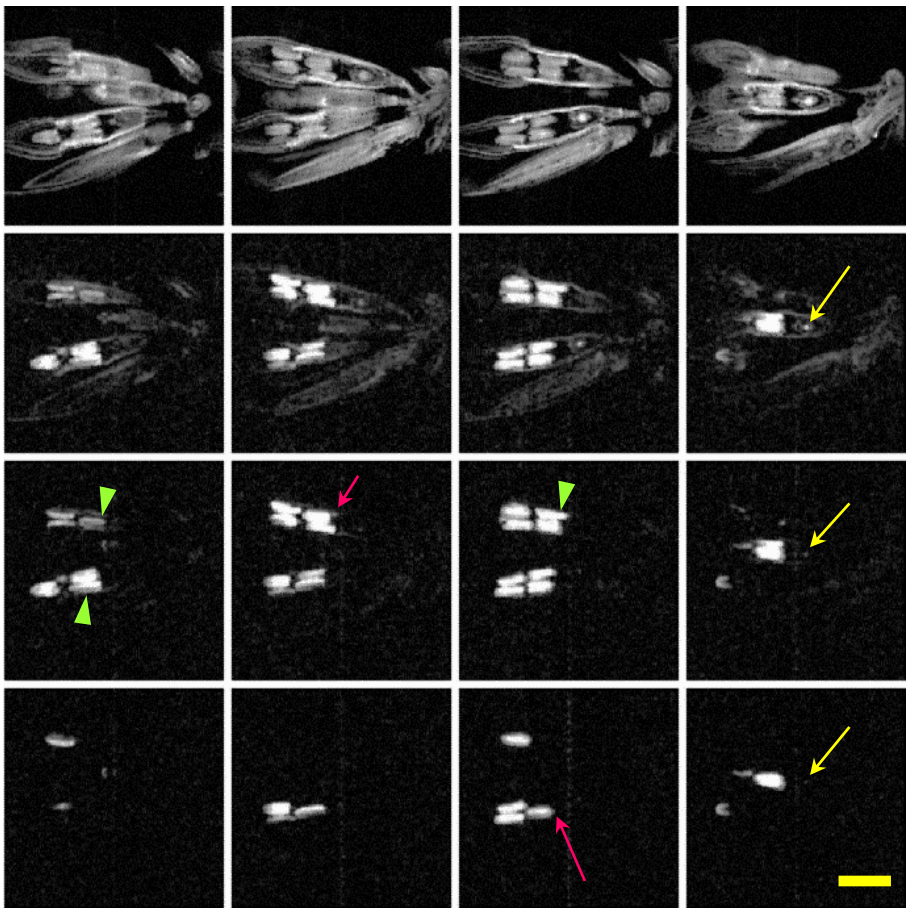

Figure 3. Longitudinal MRI images taken at +1, -7, -14, -21 °C of the same cluster of hermaphroditic *D. kamtschatica* var. *jezoensis* flower buds collected in January. Multi-slice images (4 at each time) were taken at 128 × 128 pixels and 8 scans (-7, -14, -21 °C) or at 256 × 256 pixels and 64 scans (+1 °C) per 12 × 12 mm of FOV with a slice thickness of 0.5 mm. The set of 4 slice images taken at each temperature is displayed in a row. The bud was cooled at 5 °C/h and left at the designated temperature for 10 min before taking images. Instances of embryo sacs (yellow-arrowed) and a part of anther filaments (pink-arrowed) remaining unfrozen are indicated. Some of the entire individual anthers remaining unfrozen at -14 °C (green arrow-headed) abruptly disappeared (froze) at -21 °C. The bar indicates 3 mm.

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Fig. 4A

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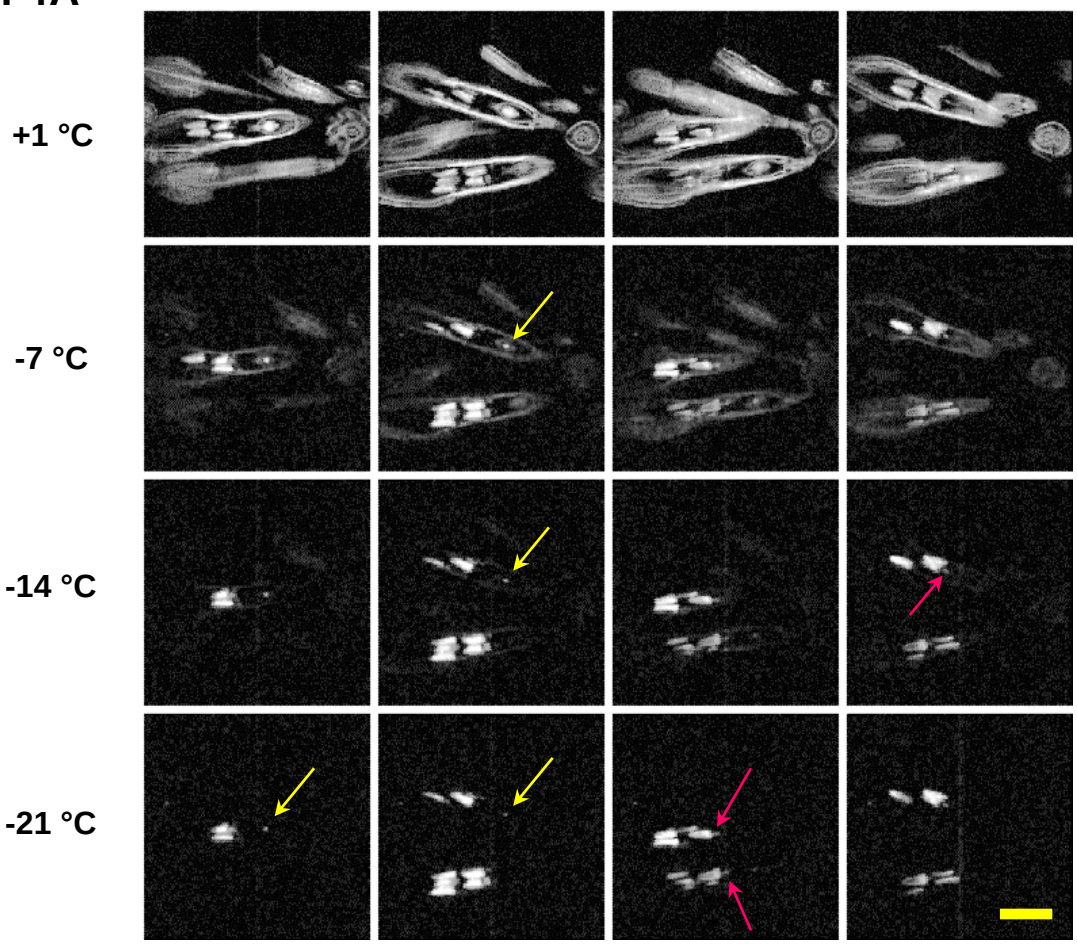

Fig. 4B

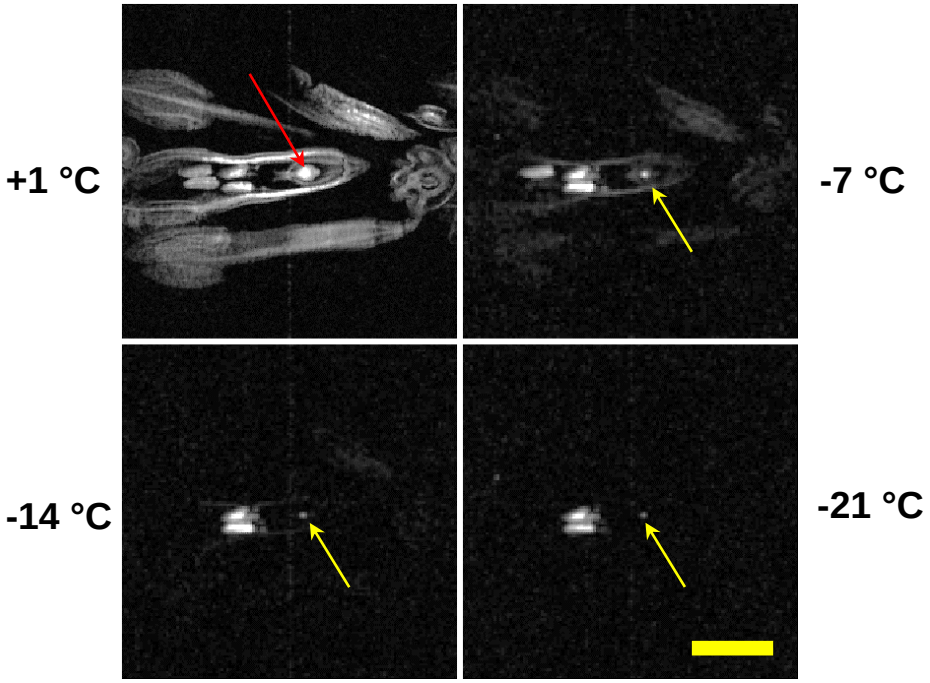

Figure 4. Longitudinal MRI images taken at +1, -7, -14, -21 °C of the same cluster of female *D. kamtschatica* var. *jezoensis* flower buds collected in January (A). Acquisition and display parameters are identical to those used for Fig. 3. The images in the extreme left column, taken at +1, -7, -14, -21 °C, are shown at higher magnification (B). The embryo sac is indicated by the yellow arrow and the ovule, by the red arrow. The bar indicates 3 mm.

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Fig. 5

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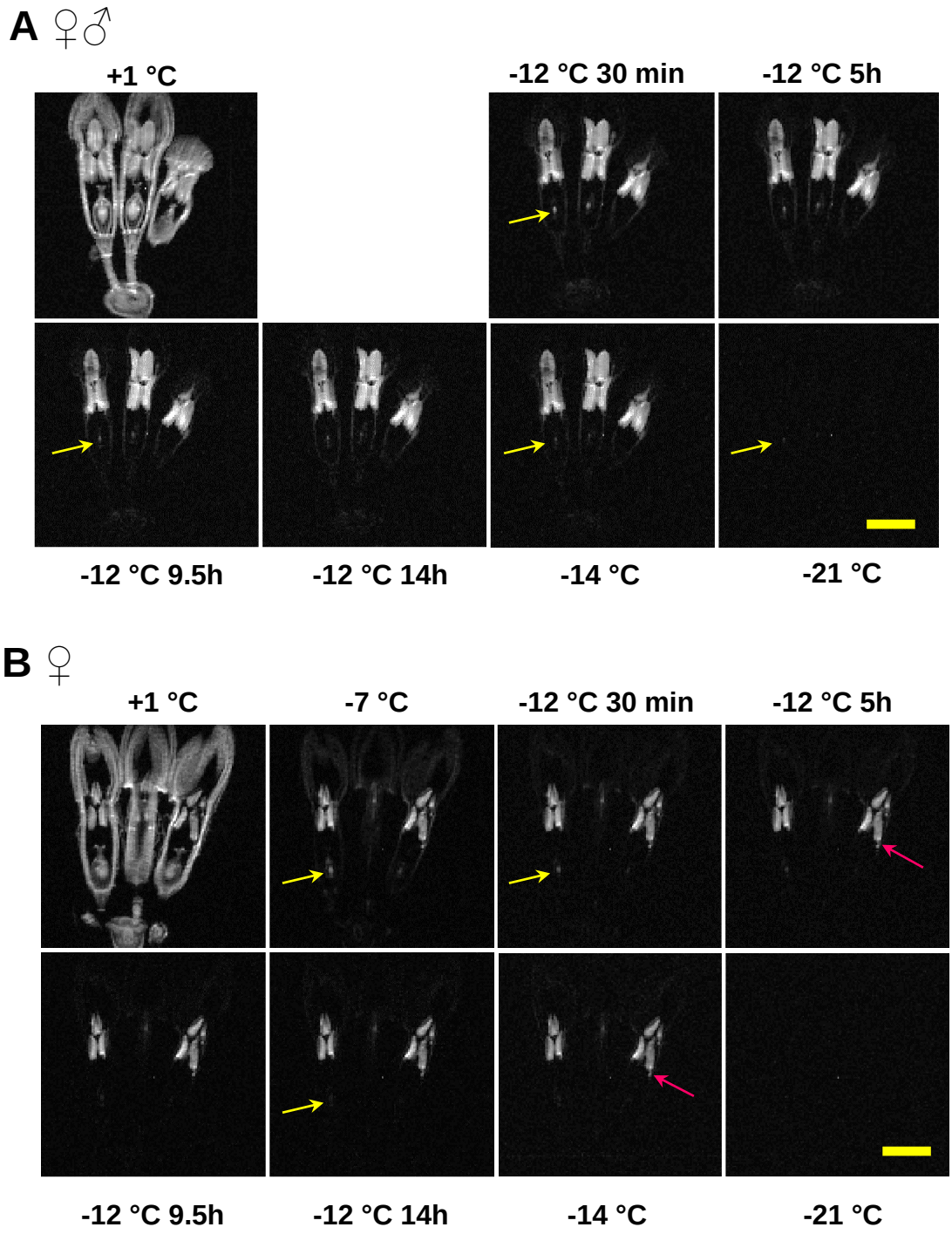

Figure 5. Longitudinal MRI images taken at +1, -12, -14, -21 °C of the same cluster of hermaphroditic (A) and female (B) *Daphne* flower buds collected in early March (slightly less cold hardy, thus the anthers all froze at -21 °C). The buds were cooled at 5 °C/h to -12 °C and kept there for 0.5 to 14 h before further cooling (5 °C/h) to -21 °C. They were left at the designated temperature for 10 min before taking images. Acquisition conditions are identical to those used for the -7 °C image in Fig. 3. Only the center slice images are shown. Instances of embryo sacs (yellow arrows) and a part of anther filaments (pink arrows) remaining unfrozen are indicated. The bar indicates 3 mm.

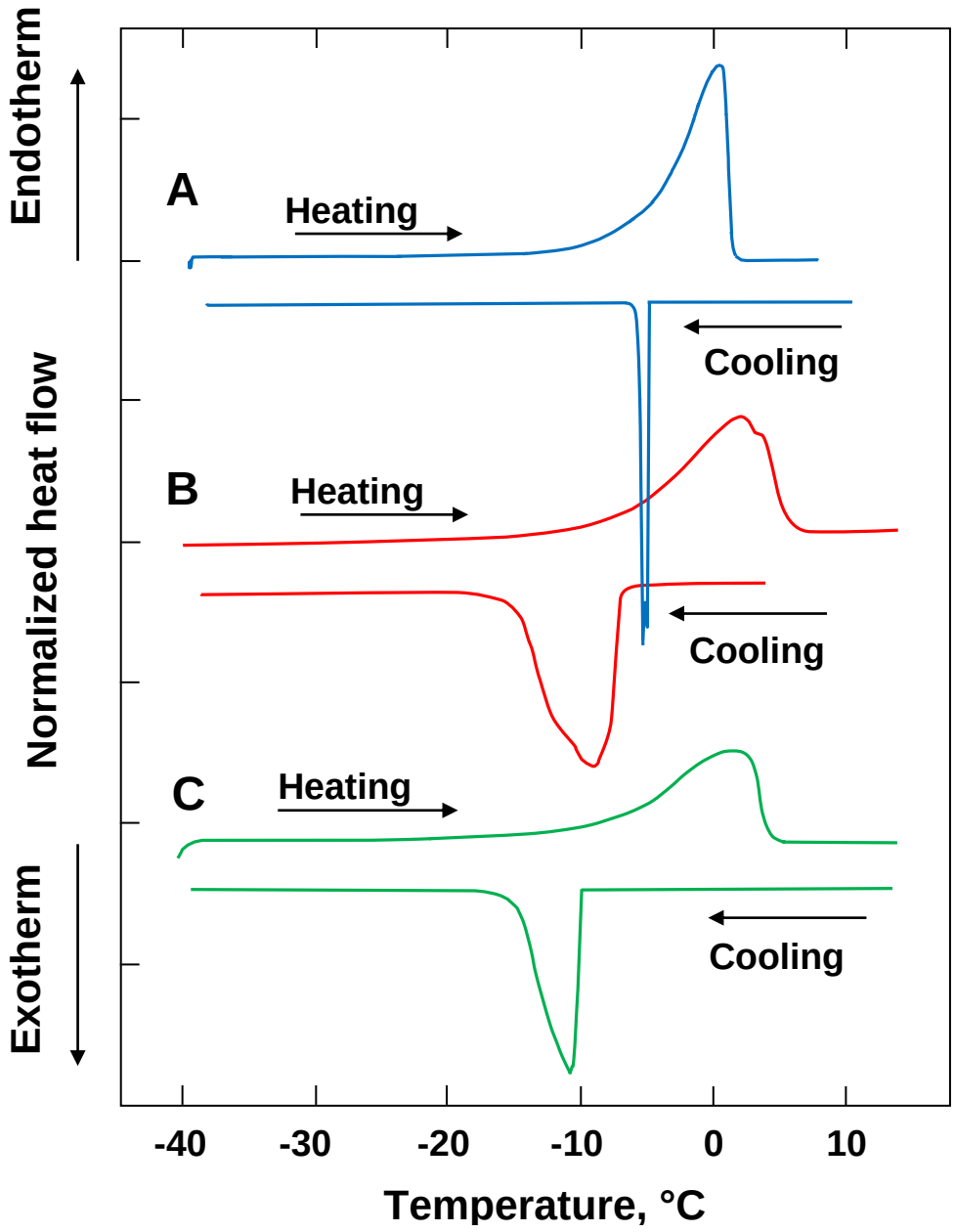

Fig. 6

Figure 6. DSC profiles of a single pistil with (A, B) or without (C) a fringe of calyx tube excised from a female flower bud of *D. kamtschatica* var. *jezoensis* collected in January The samples were cooled at either at 0.1 °C/min (A) or 5 °C/min (B, C) to -40 °C (held there for 3 min) and rewarmed at 1 °C/min (A) or 5 °C/min (B, C). The sample mass was 1.9 (A), 2.3 (B) and 1 mg (C), respectively.

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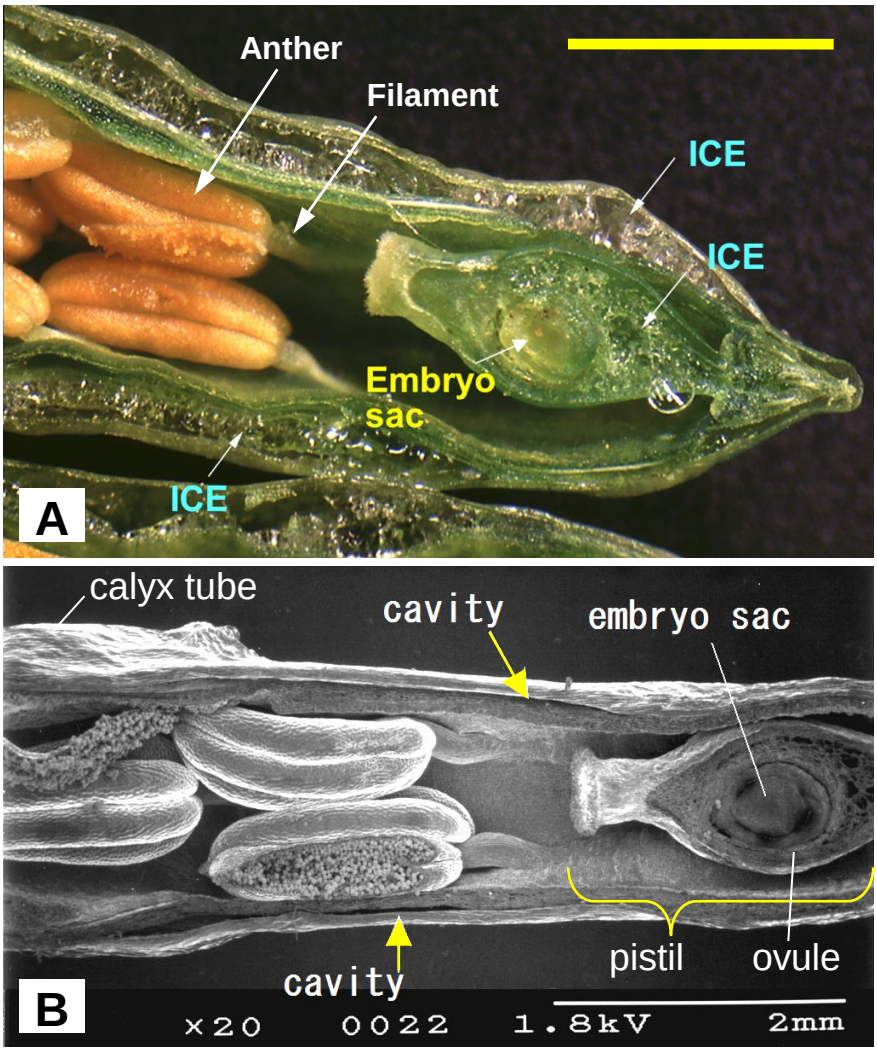

Fig. 7

Figure 7. (A) An optical microscopic view showing the localization of ice crystals in a hermaphroditic flower bud of *D. kamtschatica* var. *jezoensis* (collected in January) longitudinally dissected and observed at -10 °C following slow cooling to -10 °C. The bar indicates 2 mm. (B) A scanning electron micrograph of a longitudinally bisected *Daphne* hermaphroditic flower bud. Large cavities as a result of recurrent freeze-thaw cycles in the field is observed on the abaxial side of the calyx (or receptacle) tube. An embryo sac is in the nucellus of a single superior ovule in the pistil. Air spaces between the supporting fibrous networks around the ovule are noted. Each anther consisting of four pollen sacs enclosing pollen grains is connected to the calyx tube with a short filament.

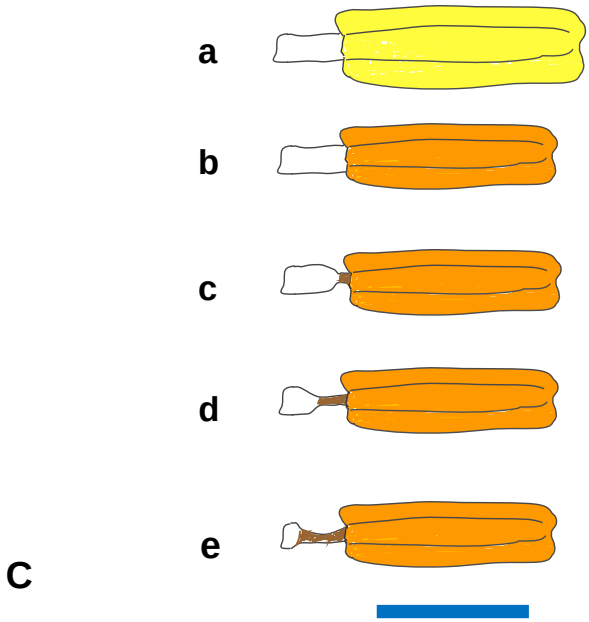

Fig. 8

Figure 8. Anther injuries of hermaphroditic (A) and female (B) flower buds of *D. kamtschatica* var. *jezoensis* (collected in January) cooled to -21 °C in laboratory freeze tests. Open and solid arrow heads indicate alive and injured anthers, respectively. The bars indicate 2 mm. (C) Illustrations showing typical freezing injury patterns (b-e) detected in anthers and their jointing filaments in hermaphroditic flower buds of *D. kamtschatica* var. *jezoensis* cooled to -15 ~ -25°C in laboratory freeze tests or in the field. A live anther with its intact filament (a) is shown as the control. The bar indicates 1 mm.

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Fig. 9

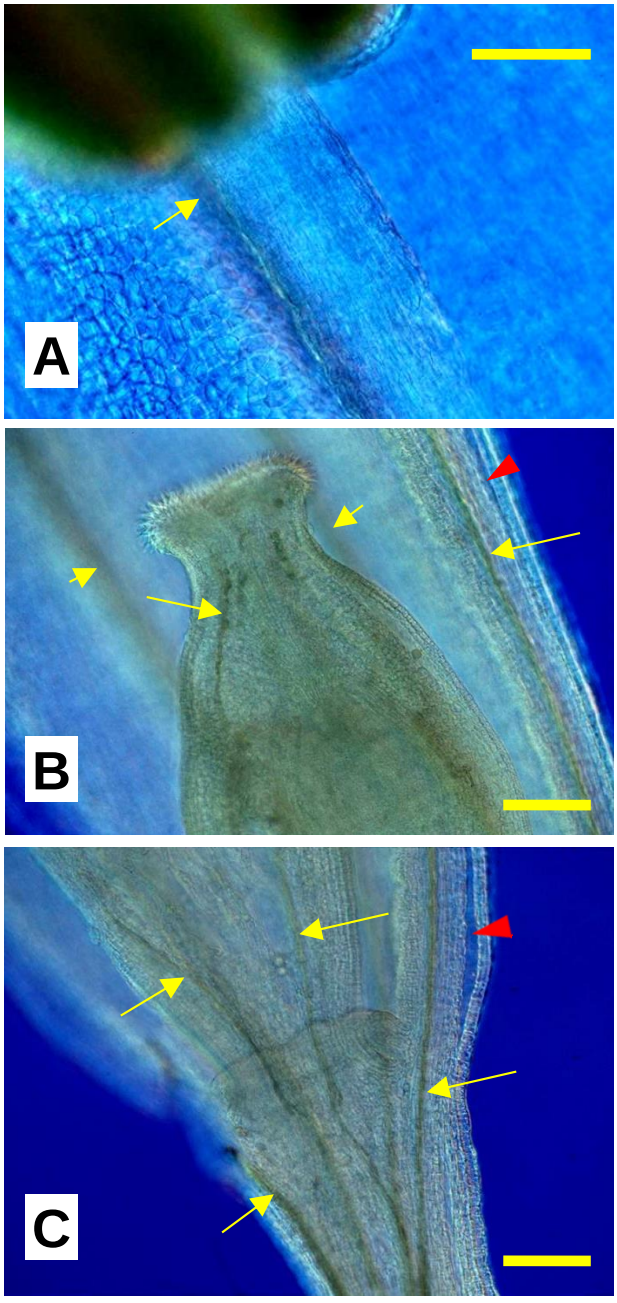

Figure 9. Cleared hermaphroditic flower bud tissues (A, an anther and its filament with the jointed calyx tube behind; B, a pistil and a calyx tube; C, the basal part of a pistil jointed to a calyx tube) of wintering *D. kamtschatica* var. *jezoensis* observed under a Nomarski microscope. Yellow arrows indicate vascular bundle networks (shown as brownish-colored thin lines) and red arrow heads, cavities created by the formation of ice crystals. The bars indicate 0.2 mm.