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PRACTICAL TOOLS

Leveraging natural history collections to understand the impacts of global change

Precision glycerine jelly swab for removing pollen from small and fragile insect specimens

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Abstract

1. Historical datasets can establish a critical baseline of plant–animal interactions for understanding contemporary interactions in the context of global change. Pollen is often incidentally preserved on animals in natural history collections. Techniques for removing pollen from insects have largely been developed for fresh insect specimens or historical specimens with large amounts of pollen on specialized structures. However, many key pollinating insects do not have these specialized structures and thus, there is a need for a method to extract pollen from these small and fragile insects.
2. Here, we propose a precision glycerine jelly swab tool to allow for the precise removal of pollen from old, small and fragile insect specimens. We use this tool to remove pollen from five families of insects collected in the late 1970s. Additionally, we compare our method with four previously published techniques for removing pollen from pinned contemporary specimens.
3. We show the functionality of the precision glycerine jelly swab for removing small quantities of pollen across insect families. We found that across the five methods, all removed pollen; yet, it was clear that some are better suited for fragile specimens. In particular, the traditional glycerine jelly swab and the precision glycerine jelly swabs both performed well for removing pollen from bee faces. The shaking wash resulted in specimen fracture and residue left behind, the ethanol rinses left setae matted, and the glycerol swabbing left residue on the specimen. Additionally, we present photographs documenting the effects of these methods on pinned honey bee specimens.
4. The precision glycerine jelly swab opens up opportunities to sample pollen from a variety of insects in natural history collections. These pollen samples can be incorporated into downstream analyses for pollen identification either via microscopy or DNA sequencing, and the resulting plant–insect interaction data can establish historical baselines for contemporary comparison. Beyond our application of this method to pollen on insects, this precision glycerine jelly swab tool could be used to explore pollen placement specialization or to sample bryophyte, fungal and tree fern spores dispersing on animals.

KEYWORDS
bees, beetles, flies, historical collection, insects, plant–insect interactions, pollen

1 | INTRODUCTION

The lack of historical data is often a limiting factor in understanding the effects of global change (Burkle et al., 2013; Hedrick et al., 2020; Moritz et al., 2008). Yet, new techniques have opened historical collections as an underutilized resource to understand the past for comparisons against contemporary data. Indeed, this has been done to great effect to characterize changes within species and has provided insights into species interactions. For instance, researchers have detected shifts in species morphology (Miller-Struttmann et al., 2015), occurrences (Bartomeus et al., 2019; Hemberger et al., 2021; Scheper et al., 2014), community composition and richness (Bartomeus et al., 2013; Fourcade et al., 2019; Rollin et al., 2020), and interactions, including herbivory (Meineke et al., 2019) and pollination services (Johnson et al., 2019; Pauw & Hawkins, 2011).

Plant–pollinator interactions are of immense importance from biodiversity, human-well-being and economic standpoints (Potts et al., 2016). There is growing concern given the documented declines of well-studied plants and pollinators (reviewed in Potts et al., 2010). Pollen analyses from museum specimens have been integral in characterizing these changes and identifying underlying drivers of decline (Kleijn & Raemakers, 2008; Scheper et al., 2014; Simanonok et al., 2021).

A crucial first step in reconstructing plant–pollinator interactions from historical specimens is to extract the pollen. Several sampling methods have been developed for extracting pollen from insects, yet the application of these methods to fragile, small, and valuable historical specimens is in its infancy (Burkle et al., 2013; Gous et al., 2019; Kleijn & Raemakers, 2008; Scheper et al., 2014; Simanonok et al., 2021). Extraction of pollen from museum insect specimens differs from fresh, field collected insects in the following ways. First, fresh insects tend to be more pliable and durable, whereas museum specimens are often dry, brittle and fragile. Furthermore, field collected insects are sometimes considered disposable following pollen extraction and insect identification. However, within the scientific community, there is a push to incorporate contemporary voucher specimens into collections (Turney et al., 2015), which would ensure the future longevity of these portals to the past. Destructive sampling should be minimized, where possible. Therefore, low-risk, non-destructive pollen extraction techniques may be vital for both field collected and historical specimens.

Much of the previous work in natural history collections has focused on extracting pollen from bees (Kleijn & Raemakers, 2008; Scheper et al., 2014; Simanonok et al., 2021). These studies focus on the pollen carried on the corbicula or scopa and use forceps or other implements to pull off a substantial amount of pollen. While undoubtedly important pollinators, bees are just one of many taxonomic groups that perform this key ecosystem service (Macgregor & Scott-Brown, 2020; Ollerton, 2017; Rader et al., 2016). One challenge to working with other animals is that they often lack specialized structures for carrying pollen. Using forceps is impractical for removing low quantities of pollen (e.g. Figure 1). Other methods, such as ethanol rinses have been used to wash pollen from fragile, 120-year-old bees (Burkle et al., 2013). Fuchsin glycerine jelly is also a standard method for removing pollen from insects (Beattie, 1971). For targeting pollen in specific areas, studies have reported using small (1–3 mm^3) cubes and entomological pins or pipette tips dipped in fuchsin glycerine jelly or sterilized glycerine (Gous et al., 2019; Macgregor et al., 2017; Motten, 1986; Traveset et al., 2015; Walton et al., 2020; Wooller et al., 1983). However, challenges exist with these other techniques: Rinsing may result in matting and loss of setae, jelly cubes can spin on the pin or be difficult to control and lead to damage of the specimens, and the dipped glycerine technique may leave a residue behind.

Given the growing interest in using historical specimens for characterizing plant–pollinator interactions, we develop and document the use of a precision gelatine swab method to extract pollen from ca. 50-year-old insect specimens. These insects spanned five families of insects, four of which lack specialized pollen carrying structures. Additionally, we provide a comparison of this new precision swab method with several of the published methods using recently pinned Western honey bee specimens *Apis mellifera*, and discuss their applicability to specimens in museum collections.

2 | METHODS

2.1 | Description of the precision glycerine jelly swab

We designed the proposed tool so that the swab material would adhere to a fine entomological pin or another implement, which allows for precision application to small areas. In preliminary trials, the commonly used ratio of 1:3:3.5 of gelatine:glycerol:water (Beattie, 1971) had difficulty adhering to the entomological pin. Thus, we prepared a precision glycerine jelly swab in a ratio of 1:1:2 of gelatine:glycerol:water. We dissolved the gelatine in water over low heat and added glycerol. If sterility is a concern for downstream applications of the pollen (e.g. DNA sequencing), we suggest that ultra-pure water be used and this solution should be autoclaved under standard conditions (e.g. 30 min at 121°C). We then allowed the solution to cool slightly, and made aliquots (5 ml) to facilitate ease of handling.

We surface sterilized entomological pins with 10% bleach and then wiped them with 70% ethanol. As we worked with small insect specimens (mean ± *SE*: 7.61 ±0.184 mm, $n = 60$, measured with the NIS-Elements D imaging software, Version 5.02), we used size 00 entomological pins. We then dipped each pin a third of the way into

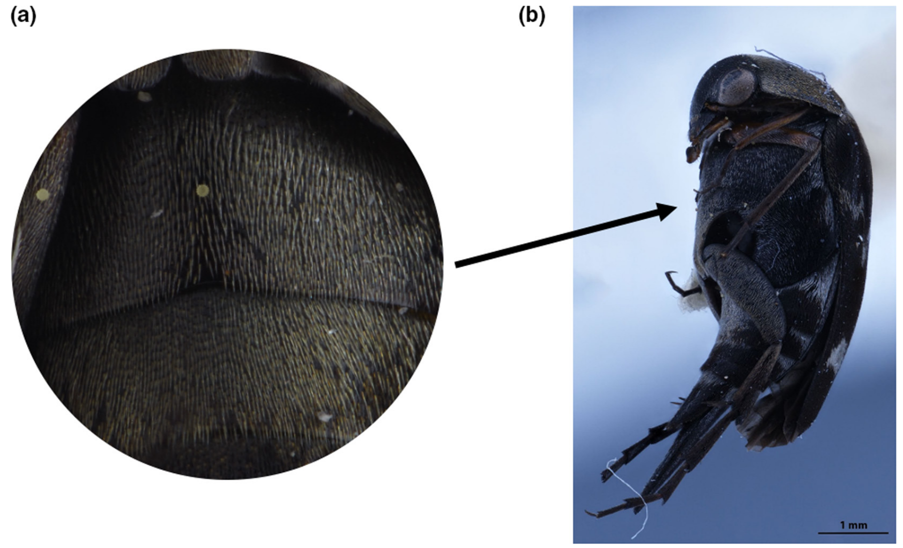

FIGURE 1 *Mordella detracta* with a close-up of pollen on its ventral aspect

the solution and removed it. We allowed the solution on the pin to set briefly. While preparing the swabs, it can be useful to use a waterbath or heatblock (*c*. 65°C) to prevent the aliquoted solution from solidifying. Depending on the viscosity of the solution (due to temperature) and the desired size of the swab, this can be repeated until the desired sized bead has formed near the tip of the pin (Figure 2). The area above the bead that was dipped will be coated in a thin layer of the solution, which will allow for sampling of narrow areas. Alternatively, prior to setting, the bead can be dragged along the lip of the solution tube to extend the coverage along the shaft of the pin, or to elongate the bead (Figure 2b). We then placed the pin swab side up in a 96-well sample rack and allowed to set for 10–20 min. Finally, we placed swabs in 2 ml microcentrifuge tubes and stored them at 4°C prior to use. This can ensure sterility, which is important for downstream work. We provide recommendations for pollen removal from the swab in Supplemental Material 1.

2.2 | Application of the precision glycerine jelly swab

We used the precision swab method on five families of insects that were collected while contacting the reproductive parts of flowers in New Zealand in the late 1970s (Primack, 1983). Families included: Syrphidae, Cerambycidae, Halictidae, Chrysomelidae and Mordellidae (two families featured: Syrphidae and Cerambycidae, Figure 3). Specifically, we viewed the pinned insect under a dissecting microscope and swabbed areas with visible pollen. For pollen that was firmly adhered to the insect, we used the pin tip to gently dislodge the pollen before passing the swab over the area. We document the condition of the insect before and after swabbing by photographing the dorsal view of the pinned insect using a Nikon AZ100M microscope with a Digital Sight DS-Ri1 (Nikon Instruments Inc.) mounted camera head. Stacked images were captured using the NIS-Elements D imaging software (version 5.02).

2.3 | Comparison with other pollen removal methods

To compare this new tool with previously published pollen sampling methods, we collected Western honey bees *Apis mellifera* foraging on *Salvia rosmarinus* in May 2021 and *Choisya ternata* in October 2021 from suburban Christchurch, New Zealand. We pinned these bees prior to pollen extraction, which is representative of how insects and pollen are presented in natural history collections. On each bee, we

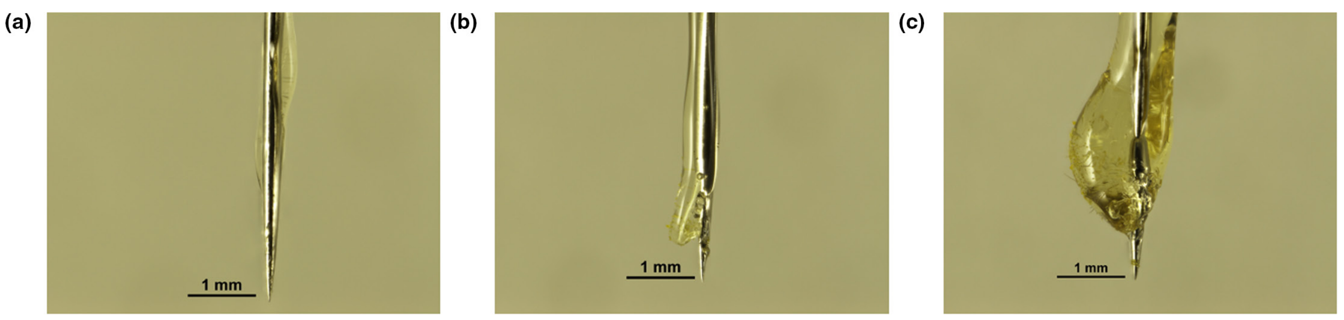

FIGURE 2 a–c are examples of the different sizes of the precision glycerine jelly swabs on 00 entomological pins. Note that b and c are post-pollen sampling and pollen and dislodged setae can be seen

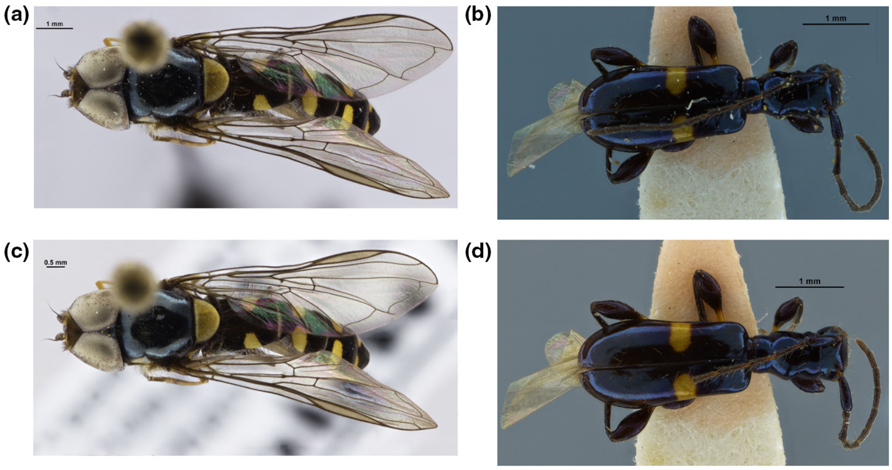

FIGURE 3 Top panels (a and b) are the insect specimens prior to pollen removal and the bottom panels (c and d) are the insect post-pollen removal with the precision glycerine jelly swab. a and c are a Syrphidae specimen and b and d are a Cerambycidae *Zorion minutum* specimen

used one of the four published methods (Table 1) or the precision gelatine swab to remove pollen from the honey bees. Each honey bee was photographed before and after pollen extraction using the same microscope, camera and imaging software as above. For pollen removal method 1, a pinned bee was placed in a 5 ml tube and 1 ml of a 1% sodium dodecyl sulphate (SDS) and 2% polyvinyl pyrrolidinone (PVP) solution was added. For method 2, we rinsed the honey bee twice with 70% ethanol, for method 3 we swabbed the abdomen of the bee with glycerol. For methods 4 and 5, we swabbed the face of the honey bee with the traditional jelly cube and the precision jelly swab while viewing the honey bee under a dissecting microscope.

2.4 | Results: Precision application

For each specimen, we were able to successfully recover most of the visible pollen. Additionally, we document the use of this technique to selectively sample pollen from specific areas of the insect (Figure 4). We show that this precision glycerine jelly swab is effective for removing pollen. In total, we used this method to sample pollen from 60 insect specimens collected in the 1970s.

2.5 | Results: Comparison of methods

The precision swabbing method is effective for extracting pollen from honey bees while minimizing morphological damage to the specimen (Figure 5j). The (a) shaking method resulted in the head, legs and abdomen separating from the thorax and a residue remained on the insect (Figure 5b). The (b) ethanol rinses resulted in matted setae (Figure 5d). The (c) glycerol swabbing method tested in the present study left residue on the insect and was also difficult to manoeuvre around the wings (Figure 5f). Overall, (d) the classic glycerine jelly swab and (e) the precision glycerine jelly swab were both effective at removing pollen (Figure 5h,j) without leaving residue, matting down the setae or severely impacting the specimen.

3 | DISCUSSION

Our goal was to develop a precision glycerine jelly swab to non-destructively remove pollen from a wide range of small and fragile insect specimens housed in natural history collections. Accessing the pollen will provide essential data for reconstructing plant–animal interactions, which may prove critical for understanding the effects of global change on these interactions.

Key advantages of the single-use precision glycerine jelly swab include ease of use, ability to target specific areas and increased manoeuvrability. The single-use nature prevents transfer among samples during processing, which may otherwise be challenging to prevent if a tool were re-used. However, future work should address possible cross-contamination of pollen during collection, processing and storage of the specimens. Based on our work, we note that care must be taken when working with insects that are

TABLE 1 Description of the five pollen removal methods that were compared using pinned honey bee specimens

No.	Method	References	Description
1	1% SDS and 2% PVP rinse	Lucas et al. (2018)	Immerse specimen in solution and shake for 1 min, allow to rest for 5 min, shake for 20 s
2	70% EtOH rinse	Kendall and Solomon (1973)*, Burkle et al. (2013)	Rinse twice, *Kendall and Solomon (1973) also brushed with camel hair brush in additon to rinsing
3	Pipette tip dipped in glycerol	Gous et al. (2019)	Swab abdomen
4	Jelly swab (1:3:3.5 gelatine:glycerol:water)	Beattie (1971), Kearns and Inouye (1993), and used extensively on fresh insects	Swab face with 2 mm^3 and 3 mm^3 cubes
5	Precision jelly swab (1:1:2 gelatine:glycerol:water)	This study	0.4 mm^3 and 1.5 mm^3 swabs on entomological pin

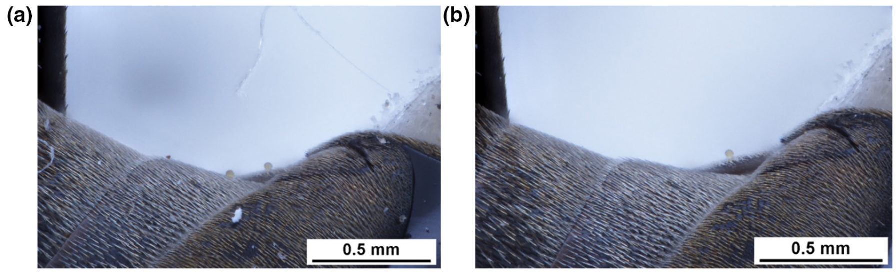

FIGURE 4 Close-up of a Mordellidae pre- (a) and post- (b) precision pollen removal targeting one but not the other pollen grain

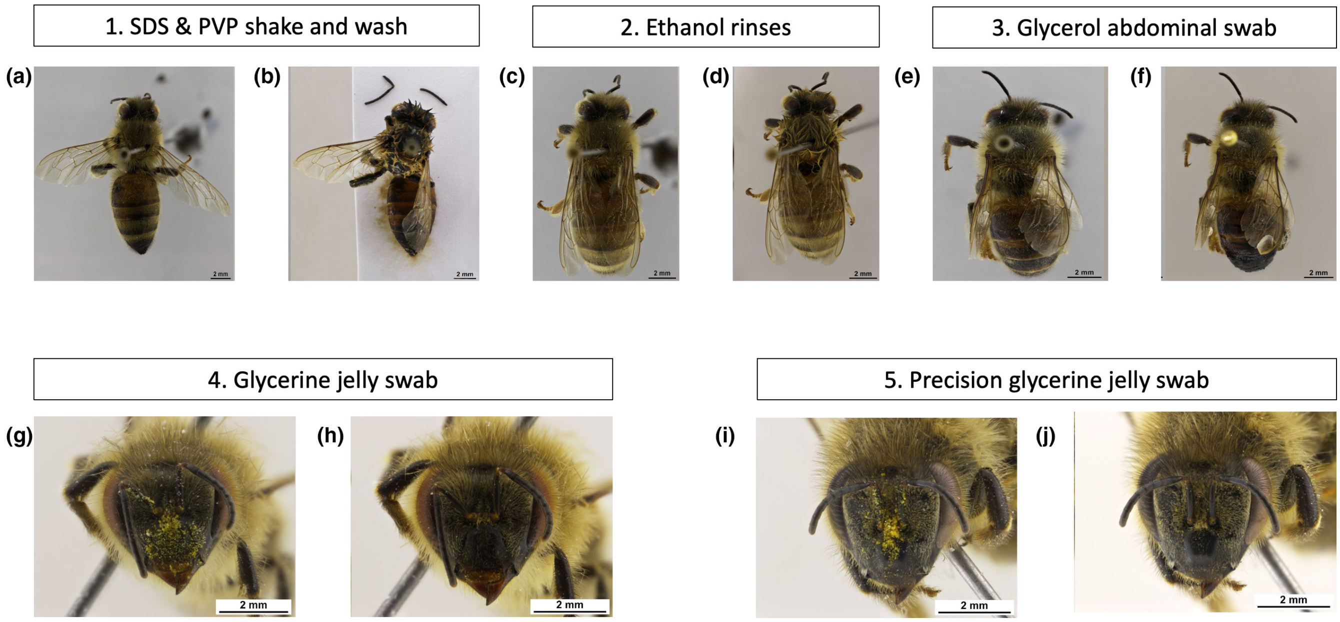

FIGURE 5 Photographs of five pinned honey bee specimens pre-pollen removal (a, c, e, g and i) and post-pollen removal with one of the four methods (b, d, f and h) or the new proposed precision glycerine jelly swab method (j)

heavily setaceous, as the setae can stick to the swab and dislodge from the insect (e.g. Figure 2c). For this reason, we suggest trialling this method on a lower value, confidently identified specimen, or a non-collection specimen, and if loss of setae is unacceptable, we recommend imaging before proceeding or the use of an alternative method. We also note that not all pollen may be able to be removed from the insect with this method. For instance, pollen that is lodged in crevices or near delicate areas, such as antennae or legs, may not be removed due to the likelihood of damaging the specimen. While this is a limitation, the ability to remove pollen from small, fragile historical specimens is an important step forward.

In our comparison of the four alternative methods, it was evident that the precision glycerine jelly swab performed well. The classic glycerine jelly swab was comparable, but despite the small size of the cube, we found that the precision swab was easier to manoeuvre around the antennae and due to its increased stickiness required fewer passes over the areas to remove pollen. While shaking in water or another solution is commonly used for fresh specimens, we strongly advise against the shaking method for dried specimens. This method and the ethanol rinse also resulted in matting of setae, and may not be appropriate for delicate, fragile bodied insects. As the glycerol swab method left residue where it was applied, it therefore may not be ideal for preservation.

Several directions for future research arise from this study. Quantification and statistical comparison of the pollen removed by each method are important. Quantification could be achieved by adding a known quantity of pollen to newly emerged honey bees and determining the amount of pollen removed by each of these methods. Furthermore, additional methods that merit future comparative study, include brushing the insects with a fine brush dipped in ethanol (e.g. Kendall & Solomon, 1973) and determining if the process for extracting insect DNA through a lysis buffer immersion (e.g. Korlević et al., 2021) is also effective for pollen. However, we add two notes of caution to the immersion approach: (a) the researchers found that pin size and placement could result in decapitation or neck extension of the insect and across the three dipteran species all lost bristles on the head and thorax during the extraction and drying procedures (Korlević et al., 2021); and (b) other recent work found decreased DNA yield associated with prolonged pollen lysis (Swenson & Gemeinholzer, 2021).

4 | CONCLUSIONS

Overall, the precision glycerine jelly swab proves useful for extracting pollen from fragile, historical insect specimens that may not have specialized pollen carrying structures. These pollen samples can easily be incorporated into downstream analyses for pollen identification either via microscopy or DNA sequencing. The method can be used to address specialization in pollen placement on both invertebrate and vertebrate animals. Beyond insects, this glycerine jelly swab method could be scaled up in relation to the size of the organisms to sample pollen from larger taxa in museum collections, such as vertebrate pollinators (e.g. birds, bats and rodents). More broadly, this tool could be used to sample fungal, bryophyte and tree fern spore dispersal on animals. Applications of this method reopen the portal to the past to understand how plant and animal interactions have changed through time, which is of particular importance given ongoing floral and faunal declines.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

N.B. developed the method; M.L.D. streamlined the methodology for use on small insects, performed the comparative methodologies and led the writing of the manuscript; and J.D.R. captured the photographs and collected insect measurements. All authors contributed critically to the drafts and gave final approval for publication.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data are available on Dryad Digital Repository https://doi.org/10.5061/dryad.rfj6q57cx (Donald et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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