TITLE: TRACKING TRENDS IN POLLINATION RATES OVER TIME USING HERBARIUM SPECIMENS OF *ASCLEPIAS SYRIACA* (COMMON MILKWEED)

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ABSTRACT:

The decline of pollinators and its potential effect on pollination service is a growing concern. The examination of herbarium specimens has proven effective in tracking pollination rates over time for certain plant species. *Asclepias* (milkweed) species require insect pollinators, and their pollen is packaged into pollinia whose removal and deposition can be readily observed in the flowers of herbarium specimens.

We investigated if there was a decline in pollination rates over time in *Asclepias syriaca* (common milkweed) by scoring the removal and deposition of pollinia for 20 flowers from each of 27 historical (collected 1862-1965) specimens and 29 recent (collected in 2009) herbarium specimens from five counties in southeastern Pennsylvania. The mean rates for pollinia deposition (0.32 ± 0.23 SD historical vs. 0.39 ± 0.32 SD recent pollinia per guiderail) were not statistically significant based on Student's T-test. The removal of pollinaria (pollinia pairs) (0.40 ± 0.24 SD historical vs. 0.55 ± 0.23 SD recent pollinaria per guiderail) was significantly higher in recent specimens, but not after controlling for flower age.

In conclusion, no evidence for a decline in pollination rates or shift in pollinator visitation over time could be detected in the southeastern Pennsylvania counties.

Tracking Trends in Pollination Rates over Time Using Herbarium Specimens of
Asclepias syriaca (common milkweed)

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INTRODUCTION

Pollinator services account for the reproduction of approximately 75% of all angiosperms (National Research Council, 2007). Many wild plants and agricultural crops such as rosaceous plants (apple, pear, cherry, plum, almond) and Arabica coffee are pollinator dependant for fruit and seed set (Biesmeijer et al., 2006; Ghazoul, 2005). Pollinator services in the United States have been valued at US$1.25 billion annually (Ghazoul, 2005). In North America, evidence of decline for the domesticated honey bee (*Apis mellifera*) has been most compelling (National Research Council, 2007). In the United States, there have been reported declines in 1947-1972, 1989-1996, and a recent drop in 2005 for managed honey bee colonies (National Research Council, 2007). A decline in pollinator populations could result in declining pollination services to pollinator dependent plants (Ashman et al., 2004). To date, however, few studies have documented a decline in pollination rates in conjunction with declines in pollinating species (Beismeijer, 2006; Pauw et al., 2010).

Biesmeijer et al. (2006) found parallel declines in bee and hover-fly assemblages and pollinator dependent plant species. Their data indicated that specialized pollinator species with narrower habitat, dietary, migratory, and developmental requirements had greater decline on average than generalist pollinator species. Their study suggested that shifts in pollinator traits such as these could lead to possible shifts in pollination services. They did not find a correlation between pollinator visitation and pollen deposition. However, shifts in plant species distribution were observed using floral inventories to see if declines in plant populations were correlated with declines in pollinator populations.

In Britain, Biesmeijer et al. (2006) found plants reliant on insect pollinators were declining on average while plants reliant on wind or water pollination were increasing. In the Netherlands, plants that were bee pollinator dependent had declined on average while plants pollinated by a wider range of pollinators had increased. Their results showed pollinator dependent plants and specialist pollinator species were declining in tandem. However, their data could not distinguish among the following alternatives: 1) plant declines preceded pollinator decline, 2) pollinator declines resulted in a loss of plant reproductive function leading to a decline in plant populations, or 3) shifts in plant and pollinator populations were responding to some other factor.

Pauw and Hawkins (2010) found parallel declines in pollination rates and populations of a South African orchid species *Pterygodium catholicum* through the comparison of historical (pre-1950) herbarium specimens to recent (post-1950) plant populations. *Pterygodium catholicum* depend heavily on the oil-collecting bee, *Redivia peringueyi,* for seed set and therefore capsule set directly relates to pollination rates across a full range of variability from 0-98% (Pearson's r= 0.99, p< 0.001, n = 15 sites, slope = 1.0) (Pauw, 2007). They found that in areas where the pollinator was absent, capsule set failed completely but where pollinator populations were abundant, higher levels of capsule set occurred.

Pauwand Hawkins (2010) detected shifts in pollination rates and populations of orchid assemblages over time and found pollination rates were higher in historical (pre- 1950)

specimens compared to recent (post-1950) pollination rates. They also showed that in urban communities where pollinator populations were less abundant, orchid assemblages were shifting to clonal species, indicating species reliant on seed propagation were declining. The examination of historical (pre- 1950) *Pterygodium catholicum* herbarium specimens therefore indicated a decline in the non-clonal orchid populations resulted from a decline in orchid pollination rates.

Here we examined 27 historical (collected 1862-1965) and 29 recent (collected in 2009) herbarium specimens of *Asclepias syriaca*, (common milkweed), from five counties in south-eastern Pennsylvania to determine if a change in pollination rates over time could be detected. Like orchids, A*sclepias* species have their pollen packaged into pollinia whose deposition and removal are detectable under a dissecting microscope. *Asclepias syriaca* is dependent on insect pollinators and is most frequently pollinated by generalist species such as, *Bombus griseocolllis* and the European honeybee, *Apis mellifera* (Kephart 1983; Kephart et al., 2003). We hypothesized that a decline in *Asclepias syriaca* pollination rates over time would correlate to a decline in the pollinator population. Herbarium specimens provide a historical record of pollination rates over time and provide consistency for the comparison of pollination rates in historical verse recent specimens.

Asclepias syriaca is in the Apocynaceae family. It has a wide range throughout North America and is commonly found in fields, along roadsides, disturbed soils, and waste grounds (USDA 2011; Rhoads & Block, 2007). According to Rhoads & Block (2007), *Asclepias syriaca* is an herbaceous perennial with erect, simple, stems, 1-2 m tall. It has opposite, oblong-lanceolate to oval leaves. It produces several umbels that are terminal and in the upper axils. It is many-flowered with a deeply divided pink-purple corolla with reflexed lobes.

Its corona consists of five prominent scoop-shaped hoods arising from near the top of the filament column with shorter horns extending from the hoods. The anthers of *A. syriaca* have a triangular appendage at the tip with erect follicles. An *Asclepias* pollinarium consists of a pair of pollinia, each attached to a translator arm that are connected by a corpusculum or "clip" (Kephart & Theiss, 2003). One pollinium develops per anther locule. The "clip" of each pollinarium can be seen with the naked eye and is situated above each of the five anther margins.

Asclepias syriaca flowering extends from June to August with flowering periods around two weeks (Kephart et al., 2003, Kephart, 1987). Most flowers are open 4-6 days with the first and and last flowers to open within each umbel opening within one day of each other in 70% of umbels (Kephart, 1987). The mean umbel size consists of 104 flowers (Kephart, 1987). Flowers in umbels produced earlier in the year remain open slightly longer than flowers in umbels produced later in the season (Kephart, 1987). The fruit develops into 3-4 inch follicles, splitting along one side to disperse its many comose seeds.

As a pollinator visits an *Asclepias syriaca* flower, the anther margin "guiderails" serve to guide an insect's appendage through the central portion of the guiderail and under the "clip" of the pollinarium (Theiss, et al., 2007, Kephart & Theiss, 2003). The insect's appendage is hooked under the "clip" and lifts the pollinarium from the anthers as it moves around or away from the

flower (Theiss, et al., 2007, Kephart & Theiss, 2003). As the insect travels around the flower or to other flowers, the anther guiderails serve to trap the pollinia within the anther margins. An insect can collect a "chain" of pollinaria via attachment of pollinaria to each other on the insect's appendages. Fertilization is then initiated once a pollinium is trapped within the anther margins.

METHODS

Field measurements of pedicel elongation during post-anthesis flower development

Five *Asclepias syriaca* flowers from each of five inflorescences were measured daily from bud to senescence for pedicel length and flower/inflorescence developmental stage. Buds on each inflorescence were individually marked with a different color paint marker on the pedicel and the pedicels were re-measured daily from June 30, 2010 to July 19, 2010. The sampling site was located at Lemon Hill in Fairmount Park, Philadelphia, Pa.

Pollination rates of herbarium specimens

Twenty-seven historical (1862-1965) and twenty-nine recent (2009) herbarium specimens were examined from Chester, Philadelphia, Northampton, Montgomery, and Bucks counties in southeastern Pennsylvania. The historical specimens were collected by various collectors from May to August and made available for this study courtesy of the Academy of Natural Sciences herbarium (PH). The recent specimens were collected by the Ann F. Rhoads, Timothy A. Block, and Lauren Spitz from June to August, 2009 and donated to PH by the Morris Arboretum herbarium (MOOR). The specimens were curated, databased, and scanned. Mention how you compared the distribution of specimen collection dates relative to the flowering season here (i.e. Fig 7).

Twenty flowers per specimen (from one to two inflorescences) were rehydrated *in situ* with damp Kimwipes® and examined *in situ* with a dissecting microscope. When a specimen had multiple inflorescences, the one or two inflorescences with the largest number of flowers at anthesis were selected. A total of 1,120 flowers were scored. For each flower, two to four anther margins "guiderails" were examined For each guiderail, the absence or presence of the pollinarium "clip" was recorded (absence=removal of the pollinarium by a pollinator). The number of pollinia deposited within each anther margin guiderails (0-3 pollinia deposited by a pollinator) was also recorded.

Pedicel lengths were digitally measured from the jpeg image using ImageJ for five of the twenty flowers scored from each specimen to control for flower age. Student's T-test was used to test the significance of the difference between the historical (1862-1965) and recent (2009) specimens of three response variables: pollinia deposition, clip removal, and pedicel length. Significance was accepted at $p=0.05$.

For each specimen, the average rate of pollinium deposition per guiderail was calculated by dividing the sum of pollinia deposited in all 20 flowers by the sum of all guiderails scored.

Likewise, the average rate of clip removal per guiderail was calculated for each specimen by dividing the sum of clips removed from all 20 flowers by the sum of all guiderails scored. The average pedicel length for each specimen was calculated for the five pedicels measured. A linear regression using pedicel length as the predictor variable and clip removal as the response variable was used to control for effect of flower age on pollination. A T-test of the residuals was used to determine if specimen age (historical versus recent) explains any additional variation not explained by flower age.

RESULTS

The field data for pedicel length measurements and flower development resulted in a correlation between pedicel length and flower development. The flower pedicel length showed to increase with flower age. The means for pedicel lengths (mm) from day one to day six are listed respectively: 23.8mm ± 3.56 SD, 25.3mm ± 3.55 SD, 26.6mm ± 3.31 SD, 27.8mm ± 4.08 SD, 28.2mm ± 4.00 SD, 30.3mm ± 4.03 SD. The average rate of elongation per day (the mean difference between pedicel lengths measured repeatedly on successive days) was 1.1mm. (See Fig. 1.)

The comparison of collection days after June 1st for the historical (1862-1965) and recent (2009) specimens resulted in the means, (37.0 ± 11.9 SD historical vs. 38.9 ± 11.9 SD recent). This difference was not significant based on Student's T-test (t= - 0.57, p = 0.57). (See Fig. 2.) The means for pollinia deposition in historical (1862-1965) vs. recent (2009) specimens resulted in (0.32 ± 0.23 SD historical vs. 0.39 ± 0.32 SD recent pollinia per guiderail). Pollinia deposition was greater in recent specimens but was not statistically significant based on Student's T-test (t= -0.96 and p= 0.34) (See Fig. 3.). The removal of clips (0.40 ± 0.24 SD historical vs. 0.55 ± 0.23 SD recent pollinia per guiderail) were higher in recent than in historical specimens and significant (t= -2.44, p=0.02) (See Fig. 4.).

The average pedicel length (2.56 ± 0.45 SD historical vs. 2.84 ± 0.31SD recent) were higher in recent than in historical specimens and also significant (t= -2.71, p=0.01) (See Fig. 5.) The regression of pollinia removed per guiderail against average pedicel length resulted in a significant and positive but weak correlation (r=0.41, p=0.002) (See Fig. 6.). After controlling for pedicel length, the residual of pollinia removed per guiderail (-0.04 ± 0.20 SD historical vs. 0.04 ± 0.20 SD recent) was not significantly different between the two groups (t= -1.42, p=0.16) (See Fig. 7.).

DISCUSSION

Flower age (Fig. 1) but not date of collection (Fig. 2) proved to be significantly different between the historical and recent specimens. Since the collectors of the recent (2009) specimens were collecting specifically for a pollination study while the collectors of the historical specimens were not, collector bias in the recent specimens is most likely the cause for the older flowers in that group. Accounting for flower age indirectly, by controlling for pedicel length,

eliminated the significant effect of this bias in our comparisons, resulting in no significant difference in pollination rates (deposition and removal) over time (Fig. 7.).

Our field data showed that pedicels elongate by an average of 1 mm per day from day 1 to day 5 of anthesis (Fig. 1.) validates the use of pedicel length as an indirect measure of flower age and pollinator exposure. Our sample for measuring the rate of pedicel elongation with flower age is limited (only one population, possible all one clone) so we don't know how precisely pedicel length predicts flower age. The correlation between flower maturity and pedicel lengths in herbarium specimens, (Fig. 5.) could be used in comparison to collection time to study phenological response to climate change over time. This area of research needs to be considered further.

No change detected in pollination rates over time indicates that no change can be detected in the total quantity of pollination service, but this does not demonstrate a change in the pollinator community. It is not possible to determine which specific pollinator species are thriving and which are declining through the observation of pollination rates alone. A comparison of the pollinator communities visiting *Asclepias syriaca* today to those recorded in past pollination studies (Kephart, 1983; Theiss, et al., 2007) may yield evidence for change in the composition of the current community.

Another avenue of research that may be pursued is the expansion of this herbarium-based method to a wider geographic range and species diversity of *Asclepias*. Perhaps the apparent stability of pollination rates we observed is particular to southeastern Pennsylvania. Pollination rates measured from herbarium specimens of rare or endangered *Asclepias* species may detect evidence of declining pollination rates over time, a potential contributing factor to the decline and displacement of species of concern (Pauw & Hawkins, 2010). In combination with pollination studies on living populations, the examination of pollination rates in herbarium specimens on a wider scale could add an important temporal dimension to the understanding of pollination change and statis.

<u>CONCLUSION</u>

The examination of *Asclepias syriaca* herbarium specimens proved effective for detecting historical pollination rates. No significant difference was found for pollinia deposition between historical and recent specimens. A significant difference in pollinaria removal between historical and recent specimens was detected but this was accounted for by a difference in flower age. No significant difference between the two groups resulted after factoring out flower age. Therefore, no evidence for change in pollination rates over time or for any decline or shift in pollinator visitation on *Asclepias syriaca* was detected in the southeastern Pennsylvania herbarium specimens.
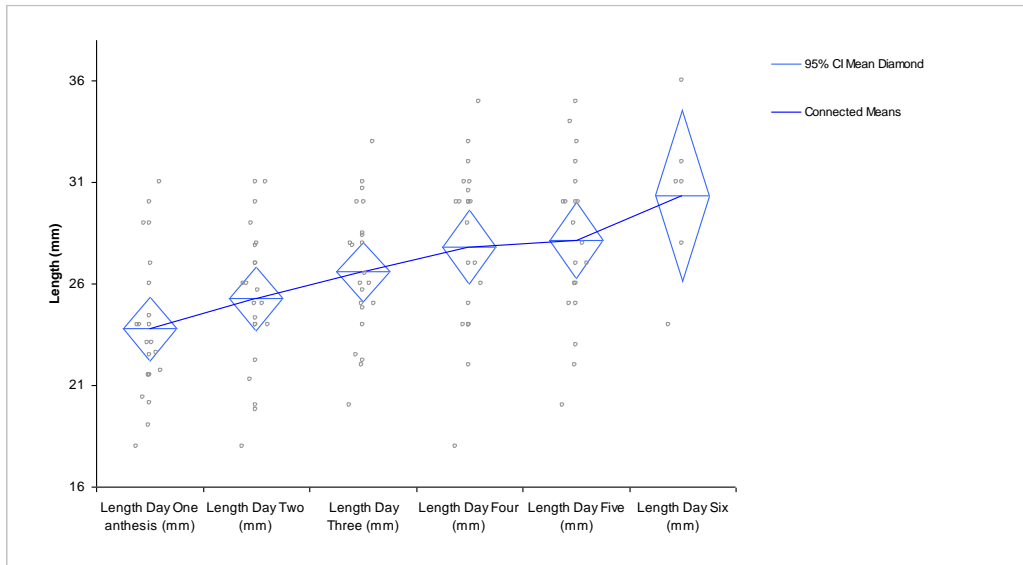
Fig. 1. Field pedicel length measurements
Elongation of individual *Aclepias syriaca* pedicels identified by inflorescence number and flower color, measured in mm (y-axis) from day 1 to day 6 of first marked flower in observed in anthesis (x-axis) based on field measurements. The means for pedicel lengths (mm) from day one to day six are listed respectively: (23.8mm ± 3.56 SD, 25.3mm ± 3.55 SD, 26.6mm ± 3.31 SD, 27.8mm ± 4.08 SD, 28.2mm ± 4.00 SD, 30.3mm ± 4.03 SD). The average rate of elongation per day (the mean difference between pedicel lengths measured repeatedly on successive days) was 1.1mm.
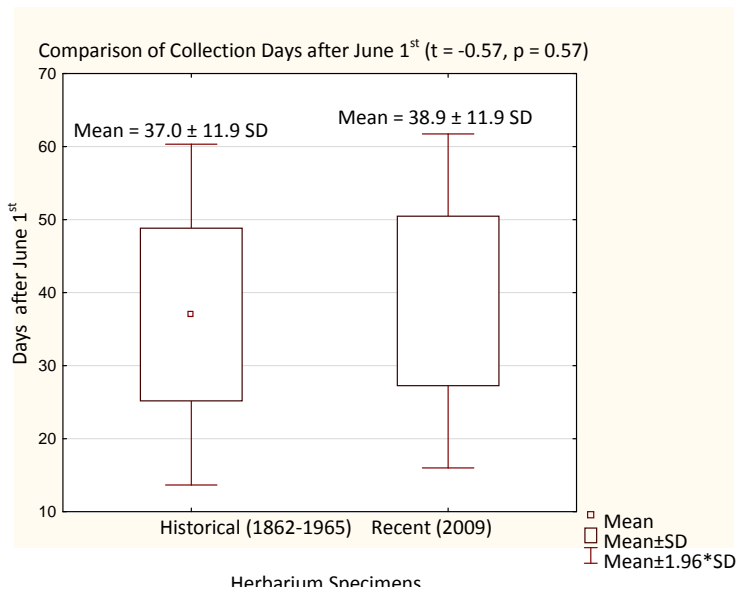
Fig. 2. Comparison of collection dates per specimen

Box graph comparing the collection times (measured as days since June 1) between historical (1862-1965) and recent (2009) specimens. The means were (37.0 ± 11.9 SD historical and 38.9 ± 11.9 SD recent) but were not significant based on Student's T-test ($t = -0.57$, $p = 0.57$).
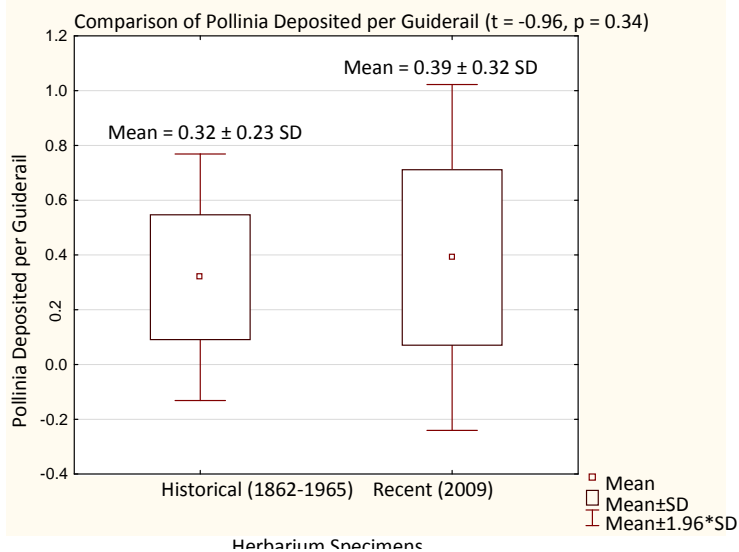

Fig. 3. Comparison of average rates for pollinia deposition per specimen

Box plot comparing the average rate of pollinium deposition per guiderail (sum of pollinia deposited for each specimen/ sum of guiderails scored per specimens). The means, 0.32 ± 0.23 SD historical versus. 0.39 ± 0.32 SD recent pollinia per guiderail, were not significantly different based on Student's T-test ($t = -0.96$ $p = 0.34$).
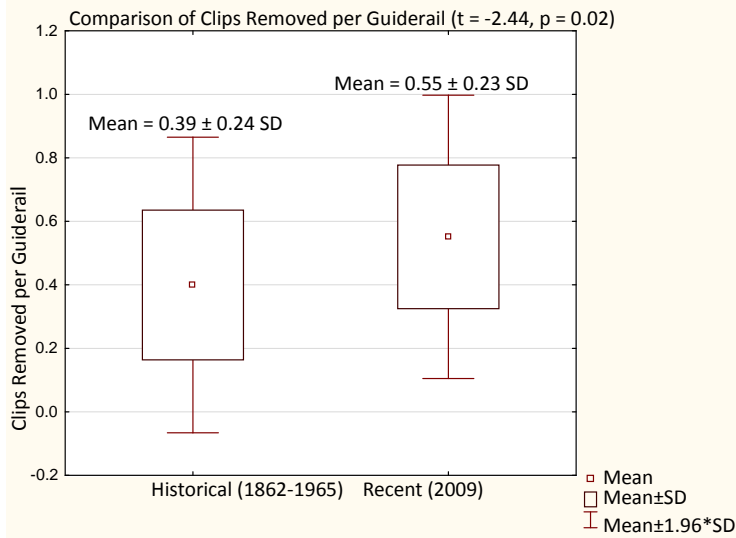
Fig. 4. Comparison of average rates for clips removed per specimen
Box graph comparing the average rate of clips removed per guiderail (sum of clips removed for each specimen/ sum of guiderails scored per specimen). The removal of pollinaria (0.39 ± 0.24 SD historical vs. 0.55 ± 0.23 SD recent pollinaria per guiderail) were higher in recent than in historical specimens, and the difference is significant (t= -2.44, p=0.02).
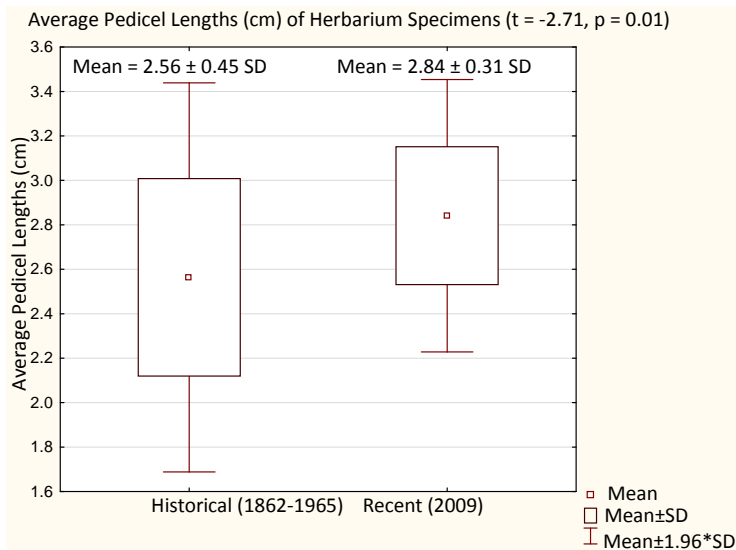

Fig. 5. Comparison of average pedicel lengths per specimen
Box graph comparing the average pedicel lengths (cm) for historical (1862- 1965) and recent (2009) herbarium specimens. Pedicel lengths were digitally measured for five of the twenty flowers scored from each specimen to control for flower age. The average pedicel length equaled the average of five flower pedicel lengths per specimen and the difference between the two groups is significant (t = -2.71, p = 0.01).
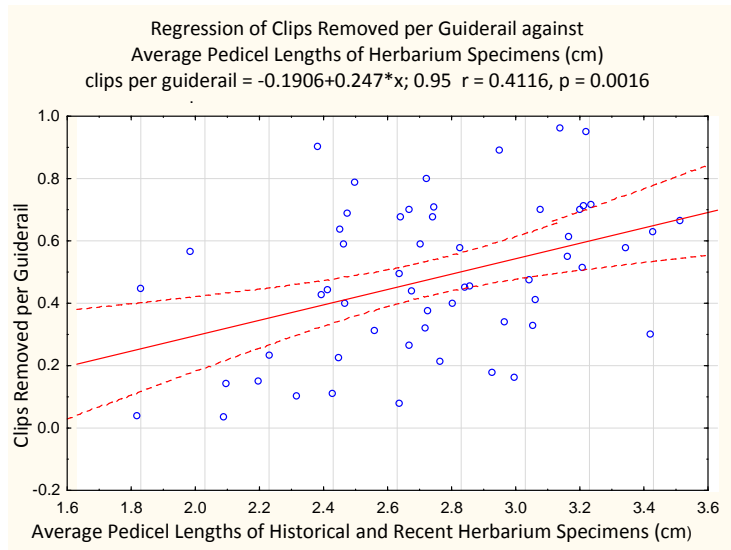
Fig. 6. Linear regression of clips removed per guiderail against pedicel length
The regression of clips removed per guiderail against average pedicel length showing a significant and positive but weak correlation (r = 0.41, p = 0.002). Herbarium specimens are represented by the blue dots and the regression line by the solid red line.
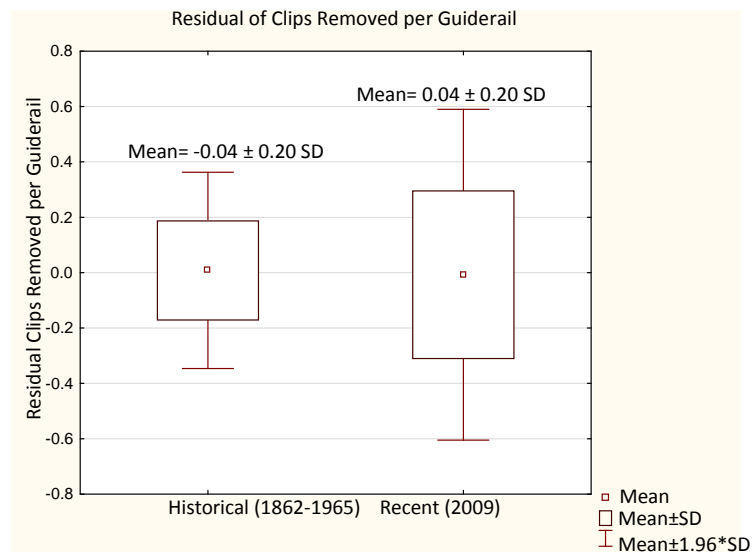

Fig. 7. Average residual rate of clips removed per specimen
Box graph comparing the residual for the average rate of clips removed per guiderail (-0.04 ± 0.20 SD historical vs. 0.04 ± 0.20 SD recent) resulting in no significant difference between the two groups (t= -1.42, p=0.16).

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