

Effect of oven-drying preservation on DNA recovery from butterfly species (Lepidoptera)

Rakesh B Patil¹, Vitthal R More²

¹ Department of Zoology, MVP'S Arts, Commerce and Science College, Dindori (Nashik) Maharashtra, India

² Department of Zoology, Government College of Arts and Science, Chhatrapati Sambhajnagar, Nagar, Maharashtra, India

Abstract

Butterflies are important components of terrestrial biodiversity and are widely used in taxonomic, ecological and molecular studies. Preservation of butterfly specimens is necessary because of their seasonal occurrence and short adult life span; however, preservation conditions may influence DNA recovery. The present study investigated the effect of oven-drying preservation on DNA recovery from five butterfly species, namely *Catopsilia pomona*, *Eurema hecabe*, *Phalanta phalanta*, *Ariadne merione* and *Danaus chrysippus*. Specimens were collected from Godapark, Gangapur Road, Nashik, Maharashtra. Fresh and oven-dried specimens were compared. DNA was isolated from 50 mg tissue using the phenol-chloroform method and quantified using the diphenylamine (DPA) colorimetric method. Mean DNA yields from fresh specimens were 96.60, 36.67, 25.00, 46.67 and 93.33 µg, respectively, whereas preserved specimens yielded 26.60, 36.67, 30.00, 23.33 and 43.33 µg, respectively. Reported DNA degradation ranged from 19.3% to 74.0%. The reported t-test values exceeded the critical value for all five species. The study indicates that oven drying can influence DNA recovery and that fresh specimens are preferable when higher DNA yield is required for molecular investigations.

Keywords: Butterflies, Lepidoptera, DNA extraction, DNA preservation, oven drying, DNA degradation, molecular study.

Introduction

Butterflies belonging to the order Lepidoptera are among the most diverse and conspicuous insects. Their characteristic wing colouration and patterns are useful for taxonomic identification and are also associated with genetic variation. Butterflies are therefore important subjects for biodiversity, ecological, evolutionary and molecular studies. DNA isolation is an essential preliminary step for molecular investigations; however, the quantity and integrity of DNA can be affected by specimen preservation. Butterflies have relatively short adult life spans and seasonal occurrence, making preservation necessary for long-term studies. Oven drying is a convenient method for maintaining external morphology, but exposure to elevated temperature may influence DNA recovery. The present study was therefore undertaken to compare DNA recovery from fresh and oven-dried butterfly specimens.

Objectives

1. To collect and preserve selected butterfly species.
2. To isolate DNA from fresh and preserved specimens.
3. To quantify DNA using the DPA method.
4. To compare DNA recovery between fresh and oven-dried specimens.
5. To determine whether the difference in DNA recovery is statistically significant.

Materials and Methods

Study Area and Collection-

Butterfly specimens were collected from Godapark, Gangapur Road, Nashik, Maharashtra, using an insect net. Collected specimens were transferred to killing jars containing chloroform and subsequently identified using external morphological characters such as wing colour, wing pattern, wing shape, margins, cell markings and other diagnostic features.

Selected Species-

Family	Species	Common name
Pieridae	<i>Catopsilia pomona</i>	Common Emigrant
Pieridae	<i>Eurema hecabe</i>	Common Grass Yellow
Nymphalidae	<i>Phalanta phalanta</i>	Common Leopard
Nymphalidae	<i>Ariadne merione</i>	Common Castor
Nymphalidae	<i>Danaus chrysippus</i>	Plain Tiger

Preservation-

Specimens were killed using chloroform, pinned and spread on spreading boards. The wings were positioned using butter-paper strips and pins. For dry preservation, specimens were placed in an oven at approximately 50°C.

DNA Extraction-

Approximately 50 mg tissue was homogenized in liquid nitrogen and treated with Lifton buffer, sodium acetate, phenol and chloroform alcohol. Following centrifugation, DNA was precipitated using chilled isopropanol, washed with 70% ethanol and air dried. The extracted DNA was subsequently used for quantification.

DNA Quantification-

DNA was quantified using the diphenylamine colorimetric method. DNA standards of 20, 40, 60, 80 and 100 µg were prepared. Samples and standards were treated with DPA reagent, heated in a boiling water bath and the resulting colour intensity was measured using a colorimeter.

Results and Observation

DNA was successfully isolated from all five selected species. The mean DNA recovery obtained from fresh and preserved specimens is presented in Table 1.

Table 1: Comparative DNA recovery from fresh and preserved butterfly specimens

Sr. No.	Butterfly species	Fresh DNA (µg)	Preserved DNA (µg)	Difference (µg)
1	<i>Catopsilia pomona</i>	96.60	26.60	70.00
2	<i>Eurema hecabe</i>	36.67	36.67	0.00
3	<i>Phalanta phalanta</i>	25.00	30.00	-5.00
4	<i>Ariadne merione</i>	46.67	23.33	23.34
5	<i>Danaus chrysippus</i>	93.33	43.33	50.00

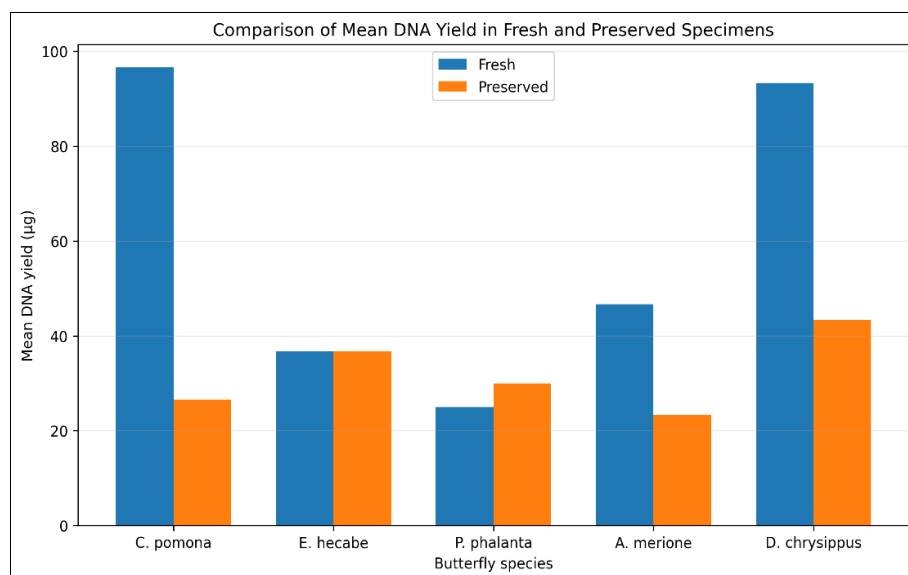
**Fig 1:** Comparison of mean DNA yield

Figure 1 compares the mean DNA yield obtained from fresh and preserved specimens of the five-butterfly species. *Catopsilia pomona* showed the highest DNA recovery in fresh specimens (96.60 µg), followed by *Danaus chrysippus* (93.33 µg). The lowest fresh DNA recovery was observed in *Phalanta phalanta* (25.00 µg). After preservation, *Danaus chrysippus* showed the highest mean DNA yield (43.33 µg),

whereas *Ariadne merione* showed the lowest (23.33 µg). A pronounced reduction in DNA recovery was observed in *C. pomona*, *A. merione* and *D. chrysippus*. In contrast, *E. hecabe* showed the same reported mean value in both conditions, while *P. phalanta* showed a slightly higher reported value after preservation.

Table 2: Reported DNA degradation following preservation

Sr. No.	Butterfly species	DNA degradation (%)	Interpretation
1	<i>Catopsilia pomona</i>	74.0	Highest
2	<i>Eurema hecabe</i>	19.3	Lowest
3	<i>Phalanta phalanta</i>	37.7	Moderate
4	<i>Ariadne merione</i>	50.0	High
5	<i>Danaus chrysippus</i>	56.3	High

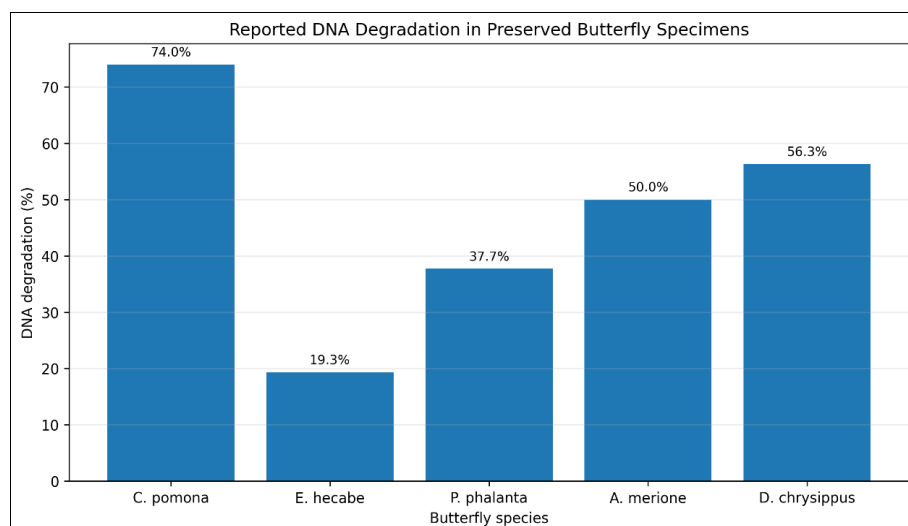
**Fig 2:** Reported DNA degradation

Figure 2 illustrates the reported percentage of DNA degradation among the five-butterfly species following preservation. The highest degradation was recorded in *C. pomona* (74.0%), followed by *D. chrysippus* (56.3%) and *A. merione* (50.0%). *P. phalanta* showed 37.7% degradation, whereas *E. hecabe* showed the lowest reported degradation (19.3%). The variation indicates that the response to preservation was species-specific.

Table 3: Statistical comparison between fresh and preserved specimens

Butterfly species	Calculated <i>t</i> -value	Critical value	Statistical result
<i>C. pomona</i>	4.0853	3.747	Significant
<i>E. hecabe</i>	10.33	3.747	Significant
<i>P. phalanta</i>	-8.25	3.747	Significant
<i>A. merione</i>	5.03	3.747	Significant
<i>D. chrysippus</i>	20.18	3.747	Significant

The comparative analysis demonstrates considerable variation in DNA recovery among the investigated butterfly species. Fresh specimens generally showed greater DNA recovery, particularly in *C. pomona* and *D. chrysippus*. The reduction following preservation was greatest in *C. pomona*, whereas *E. hecabe* showed no difference between the reported fresh and preserved mean values. The reported degradation values further indicate that preservation affected species differently, with degradation ranging from 19.3% to 74.0%.

The statistical analysis reported significant differences between fresh and preserved specimens for all five species because the calculated *t*-values exceeded the reported critical value of 3.747. These findings suggest that preservation conditions, particularly oven drying, should be carefully considered when butterfly specimens are intended for subsequent DNA-based molecular investigations.

Discussion

The results indicate that preservation influenced DNA recovery in the selected butterfly species. A considerable reduction in DNA yield was observed in *C. pomona*, *A. merione* and *D. chrysippus*. The study suggests that exposure to approximately 50°C during oven drying may contribute to DNA degradation. However, the response varied among species, as shown by the relatively small, reported difference in *E. hecabe* and the higher preserved value recorded for *P. phalanta*.

The difference between fresh and dried tissue should also be interpreted in relation to water loss during drying. The source study reported approximately 66% water content for *E. hecabe* and discussed how equal tissue weights do not necessarily represent equal amounts of biological material after drying.

Overall, the findings suggest that oven drying can affect DNA recovery and should be selected cautiously when preserved specimens are intended for molecular studies.

Conclusion

The present study demonstrates that preservation method is an important factor influencing DNA recovery from butterfly specimens. Fresh specimens generally provided higher DNA yields than oven-dried specimens, although species-specific differences were observed. The reported DNA degradation ranged from 19.3% to 74.0%, and the

statistical analysis indicated significant differences between fresh and preserved samples. Therefore, fresh specimens are preferable when higher DNA recovery is required for molecular investigations, while alternative preservation methods should be evaluated for long-term specimens intended for genetic and DNA-based studies.

Future Scope

Further studies should compare oven drying with silica-gel preservation, ethanol preservation, freezing and other low-temperature methods. DNA concentration should also be supplemented with DNA purity, integrity, PCR amplification and DNA-barcoding analyses to determine the actual molecular suitability of preserved specimens.

References

1. Brower AVZ. Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. *Proc Natl Acad Sci*,1994;91:6491-5.
2. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem J*,1955;62:315-23.
3. Calderon-Cortes N, Quesada M, Cano-Camacho H, Zavala-Paramo G. A simple and rapid method for DNA isolation from xylophagous insects. *Int J Mol Sci*,2010;11:5056-64.
4. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem*,1987;162:156-9.
5. Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN. A minimalist barcode can identify a specimen whose DNA is degraded. *Mol Ecol Notes*,2006;6:959-64.
6. Hundsdoerfer AK, Kitching IJ. A method for improving DNA yield from century-plus old specimens of large Lepidoptera while minimizing damage to external and internal abdominal characters. *Arthropod Syst Phylogeny*,2010;68:151-5.
7. Kunte K. Butterflies of Peninsular India. India: Universities Press.
8. Mtambo J, Bortel WV, Madder M, Roelants P, Backeljau T. Comparison of preservation methods for reliable DNA amplification by PCR. *Exp Appl Acarol*,2002;38:189-99.
9. Nijhout HF. The Development and Evolution of Butterfly Wing Patterns. Smithsonian Institution Press.
10. [S, Manickam A. Biochemical Methods for Agricultural Sciences. Wiley Eastern Limited.