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Use of DNA sequences to identify forensically important fly species and their distribution in the coastal region of Central California

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ABSTRACT

Forensic entomology has gained prominence in recent years, as improvements in DNA technology and molecular methods have allowed insect and other arthropod evidence to become increasingly useful in criminal and civil investigations. However, comprehensive faunal inventories are still needed, including cataloging local DNA sequences for forensically significant Diptera. This multi-year fly-trapping study was built upon and expanded a previous survey of these flies in Santa Clara County, including the addition of genetic barcoding data from collected species of flies.

Flies from the families Calliphoridae, Sarcophagidae, and Muscidae were trapped in meat-baited traps set in a variety of locations throughout the county. Flies were identified using morphological features and confirmed by molecular analysis. A total of 16 calliphorid species, 11 sarcophagid species, and four muscid species were collected and differentiated. This study found more species of flies than previous area surveys and established new county records for two calliphorid species: *Cynomya cadaverina* and *Chrysomya rufifacies*. Differences were found in fly fauna in different areas of the county, indicating the importance of microclimates in the distribution of these flies. Molecular analysis supported the use of DNA barcoding as an effective method of identifying cryptic fly species.

1. Introduction

Accurate species identification and up-to-date locality information are essential for the effective application of forensic entomology in criminal investigations, and an ever growing body of research has shown molecular data to be one of the fastest and most reliable methods of accomplishing species-level identification [1,2]. Historically, a 304 bp sequence of the COI region of mtDNA was used for calliphorid identification [3]; more recent forensic studies have utilized the 658 'barcode' COI fragment [4–7]. The relatively low cost of DNA sequencing and reproducibility of PCR methodologies has generated a large body of molecular data, increasing the need for a comprehensive set of reference DNA for forensic flies [8]. Researchers can compare sequences of their evidence item to reference sequences in a databank of published DNA sequences such as GenBank, use a sequence alignment tool (i.e. BLAST), and compare genetics distance. While comparative matching of sequences is quickly gaining favor, it should be noted that care should be used in completely relying on sequence data alone. For example, Dawnay, Ogden, McEwing, Carvalho, and Thorpe [9] tested the effectiveness of checking experimentally sequenced DNA against data in GenBank for use in forensic applications, and found that although the essential methodology and concept behind the model was sound, the value in real-life situations was limited by the accuracy and completeness of the reference DNA sequence collection. One troubling example involved a human DNA sample which produced a 100% match with five invertebrate species in GenBank, implying that compromised sequences existed in the reference collection, likely due to contamination that occurred during the original sequence submissions [9]. Park et al. [10] cites a fly from China identified as *Aldrichina grahami* in GenBank, whose DNA sequence diverged significantly (6.5–6.9% sequence distances) from both their own sequences from the same species in Korea and other sequences of *A. grahami* within GenBank. The authors found this fly matched up closely (0.7–1.4% sequence distance) to their *Calliphora vicina* samples, suggesting the Chinese fly in GenBank had most likely been misidentified. Thus, while the need for more banked sequences is imperative, more rigorous standards for sequence submission may be essential in the future to preserve data integrity.

As the body of GenBank data grows, so may the temptation to simply rely on published material when it becomes available

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Table 1
Descriptions of standardized fly trapping sites.

Site Name	Latitude	Longitude	City	Habitat type[a]
Sunnyvale	37°21′56″N	122°00′43″W	Sunnyvale	Urban
Alum Rock Park	37°23′52″N	121°47′59″W	San José	Coastal Scrub/Coastal Oak Woodland
Grant Ranch	37°20′35″N	121°42′56″W	San José	Coastal Oak Woodland/Unknown Shrub Type
San José State University	37°20′09″N	121°52′49″W	San José	Urban
Skyline	37°13′50″N	122°05′44″W	Saratoga	Redwood/Unknown Conifer Type

[a] Habitat types are as categorized by the California Department of Forestry and Fire Protection's Fire and Resource Assessment Program (FRAP) [23].

rather than proactively conduct regional surveys to collect and submit sequences from local fly specimens. Stevens and Wall [11] and Wells and Williams [12] both stress the need for more original data from localities worldwide, both to refine identifications down to the subspecies level and to account for variations in different geographic regions. Wells and Williams emphasized the importance of considering non-genetic information, such as natural history, seasonality, or geographic distribution, when using molecular techniques for identification. In a study using COI to identify calliphorids in the genus *Lucilia*, Wells, Wall, and Stevens [13] found that whereas *L. cuprina* and *L. sericata* have distinct COI haplotypes in most parts of the world, they were not able to definitively distinguish samples in Taiwan using this gene. They also noted that while *L. illustrus* and *L. caesar* have very similar COI patterns, the fact that only *L. illustrus* is known to occur in the New World significantly improves the utility of the molecular data. More recently, DeBry, Timm, Wong, Stamper, Cookman, and Dahlem found that two species: *Lucilia coeruleiviridis* and *L. mexicana*, statistically share the same COI sequence, although their ranges appear divergent [14].

In Central California, local DNA reference data for the three most forensically important families (Calliphoridae, Sarcophagidae, and Muscidae) and information on biodiversity, abundance, seasonality, and natural history are either scarce or completely nonexistent [15]. The most comprehensive California fly distribution information for calliphorids and muscids is found in James [16] and Eldridge [17], respectively, from museum-collected specimens. Brundage, Bros, and Honda [18] offered a more recent assemblage of the regional calliphorid fauna, and while this study provided seasonality and information on calliphorid diversity it was not comprehensive, did not utilize molecular data, and did not address other families of forensically important flies in the area. Niemela [19] most recently studied the distribution of forensically important flies using museum specimens only.

In this study, we trapped forensically significant flies over a number of seasons primarily in Santa Clara County using a variety of bait types. Our objectives were threefold: 1) to develop a thorough taxonomic and ecological inventory of the forensically important flies in the Central California region, 2) determine the utility of DNA sequences as an accurate species identifier for forensically important flies in the region, and 3) continue to add voucher-based sequence information from a geographic region that has not been extensively sampled.

2. Materials and methods

2.1. Pilot studies 2005–2009

During this five-year period, we utilized a number of traps and collecting methods to capture flies in both urban and rural areas of Santa Clara, Santa Cruz, and San Mateo Counties. Flies were collected using a combination of methods, including homemade soda bottle traps as constructed per Honda [15], sweep-netting, and one instance of a slightly modified CDC Gravid Trap Model 1712 (John W. Hock Company, Gainesville, FL) deployed for a week over a cardboard box containing a crow carcass. Store-bought insect traps of the type used previously by Brundage et al. [18] were field-tested but did not perform as well as the homemade bottle traps.

The previous local fly survey relied solely upon beef liver mixed with water [18]. The literature provides a wide assemblage of bait examples, from pork liver and raw squid [10] to whole rabbit carcasses [20,21]. For this project, several different bait types were tested, including fish and cuts of chicken, turkey, pork and beef in varying stages of decay. While not quantified, it was determined that the homemade bottle traps baited with rancid fish captured a relatively high volume and diversity of flies over a 24-h period.

In conjunction with the trapping efforts, from 2005 to 2007, 2–4 bottle traps were set per week at urban and rural sites primarily during the summer, when the largest assortment of calliphorid species have been collected [22]. Trapping was conducted sporadically basis in spring, fall, and winter.

The combination of these trapping efforts, supplemented by opportunistic hand-collecting of observed specimens, was successful for completing the collection of most of the historically recorded species of calliphorids in the region. Moreover, some of the traps placed in two rural areas (Grant Lake and Skyline) captured a number of flies not previously collected in the area by Brundage et al. [18]. A number of unidentified sarcophagids and muscids were collected from these traps. Standardized trapping sites are listed in Table 1 and pictured in Fig. 2. A small number of flies that were trapped or swept from single-occurrence collection sites in Santa Clara or San Mateo County were also included in portions of this study.

2.2. Survey 2010–2011

Following the pilot studies, a 12-month survey was conducted from July 2010 through June 2011. On a monthly basis, or semi-monthly basis when weather permitted, a set of at least two traps were set out at each of two diverse areas in Santa Clara County (Fig. 1): at Sanborn-Skyline County Park in Saratoga (“Skyline”), and near Grant Lake in Joseph D. Grant County Park (“Grant”) adjacent to the city of San José. These two sites were selected because 1) they are rural and would target previously uncollected/native fly species, 2) the habitats have vastly different microclimates, and 3) historically represent areas that have high numbers of disposed human remains in our previous work on human remains (personal observations).

Additionally, traps were set approximately bi-monthly at two of the most productive sites trapped previously in the first round of trapping: in the residential backyard in Sunnyvale, and at Alum Rock Park in San José. These two locations received additional trapping to ensure sufficient sampling during all seasons in these areas, as well as to test whether the use of different bait types attracted any previously uncollected species of flies.

The 2010–2011 trapping regimen had the dual purpose of starting a baseline collection of forensically significant sarcophagid and muscid species in the region, as well as capturing additional calliphorid species, as these were areas of the county not previously trapped year-round for this project. Since no previous studies have actively cataloged local sarcophagid or