Western Riverside County
Multiple Species Habitat Conservation Plan
Biological Monitoring Program

2021 Quino Checkerspot Butterfly
(Euphydryas editha quino)

April 2022

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

NOTE TO READER:

This report is an account of survey activities conducted by the Biological Monitoring Program for the Western Riverside County Multiple Species Habitat Conservation Plan (MSHCP). The MSHCP was permitted in June 2004. Reserve assembly is ongoing and is expected to take 20 or more years to complete. The Conservation Area includes lands acquired under the terms of the MSHCP and other lands that have conservation value in the Plan Area (called public or quasi-public lands in the MSHCP). In this report, the term "Conservation Area" refers to these lands as they were understood by the Monitoring Program at the time the surveys were conducted.

The Monitoring Program monitors the status and distribution of the 146 species covered by the MSHCP within the Conservation Area to provide information to Permittees, land managers, the public, and the Wildlife Agencies [i.e., the California Department of Fish and Wildlife (CDFW, formerly California Department of Fish and Game) and the U.S. Fish and Wildlife Service]. Monitoring Program activities are guided by defined conservation objectives for each Covered Species, other information needs identified in MSHCP Section 5.3 or elsewhere in the document, and the information needs of the Permittees. A list of the lands where data collection activities were conducted in 2021 is included in Section 8.0 of the Western Riverside County Regional Conservation Authority (RCA) Annual Report to the Wildlife Agencies.

The primary author of this report was the 2021 Quino Survey Lead, Esperanza Sandoval. This report should be cited as: Biological Monitoring Program. 2021. Western Riverside County MSHCP Biological Monitoring Program 2021 Quino Checkerspot Butterfly (*Euphydryas editha quino*; Quino) Survey Report. Prepared for the Western Riverside County Multiple Species Habitat Conservation Plan. Riverside, CA. Available online: https://www.wrc-rca.org/species-surveys/.

While we have made every effort to accurately represent our data and results, it should be recognized that data management and analysis are ongoing activities. Readers wishing to make further use of the information or data provided in this report should contact the Monitoring Program to ensure that they have access to the best available or most current data.

Please contact the Monitoring Program Administrator with questions about the information provided in this report. Questions about the MSHCP should be directed to the Executive Director of the RCA. Further information on the MSHCP and the RCA can be found at www.wrc-rca.org.

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INTRODUCTION

The Quino checkerspot butterfly (*Euphydryas editha quino*; Quino) is federally listed as endangered and is sparsely distributed within the southeastern section of the Western Riverside County MSHCP Plan Area. Species-specific Conservation Objective 4 states that "within the MSHCP Conservation Area, biologists will document the distribution of Quino checkerspot butterflies throughout the Plan Area on an annual basis" (Dudek & Associates 2003). Biological Monitoring Program biologists attempted to meet this objective by focusing surveys within the six Core Areas identified in Conservation Objective 1: Warm Springs Creek, Johnson Ranch/Lake Skinner, Oak Mountain, Wilson Valley, Sage, and Silverado/Tule Peak (Dudek & Associates 2003). The Lake Mathews/Estelle Mountain/Harford Springs Core Area was historically occupied by Quino but the species is now extirpated at this location (Dudek & Associates 2003) and surveys were not conducted there from 2013 through 2019 and 2021. One visit was made to the Lake Mathews/Estelle Mountain/Harford Springs Core Area to assess the habitat for Quino in 2020 (Biological Monitoring Program 2020). Additional surveys were conducted in three Satellite (non-core) Occurrence Complexes where Quino are known to currently or historically occur: the southwestern portions of the San Bernardino National Forest (SBNF), Cactus Valley, and Aguanga.

The Quino checkerspot butterfly is a member of the checkerspot *Euphydryas* complex within the brush-foot butterfly (*Nymphalidae*) family. The term "checkerspot" refers to the repeated pattern of black, cream-colored, and orange spots that are the characteristic colors of the wings (Ehrlich and Hanski 2004). A diagnostic characteristic of the adult Quino is the orange stripes (rather than white) across the top of the abdomen and the absence of white spots. Quino larvae can be recognized after their second molt by their black coloration and rows of eight to nine orange tubercles on their back (USFWS 2003). These larvae are most typically observed feeding on host plants, particularly *Plantago erecta* (California plantain). Other Quino larvae host plants include *Plantago patagonica, Collinsia concolor, Sairocarpus coulterianus, Castilleja exserta*, and *Cordylanthus rigidus* (Pratt and Pierce 2010).

The life cycle of Quino usually includes one generation of adults per year, with a four to six-week flight period (Emmel and Emmel 1973). Quino larvae come out of diapause (post-diapause larvae) around February with the emergence of host plants to feed and molt into larger instars until pupating. Quino forms their pupae low to the ground using their host plants or other vegetation as cover and remain in this stage for about ten days (Mattoni et al. 1997). Males emerge about 2-3 days before the females, once females emerge mating immediately follows. Mating occurs in early to mid-spring, generally in February (low elevation areas) and March (higher elevations) in western Riverside County. Females then lay masses of eggs in small clusters at the base of their host plants (Pratt and Emmel 2010). One or two egg clusters per day are laid for most of the butterfly's ten to 14-day adult life (Labine 1968). The egg clusters hatch in about two weeks and the newly emerged larvae (pre-diapause larvae) seek shelter on their host plant creating a web-like protective cover around them and begin feeding (Pratt and Emmel 2010). The grass- and shrub- lands that support the Quino checkerspot butterfly and its larval host plants dry rapidly in late spring, but drying may occur earlier in the absence of

sufficient autumn or winter precipitation, which is why the pre-diapause phase is the most vulnerable, and larval mortality commonly exceeds 99% (White 1974).

If host plants persist, larvae grow through three instars. As summer drought commences and their host plants senesce, they molt into a fourth instar and enter a summer diapause (Ehrlich and Hanski 2004). Quino larvae tend to seek shelter at the base of shrubs that surround the host plants, such as *Eriogonum fasciculatum* (Pratt and Emmel 2010). The larvae that successfully entered diapause will remain in this dormant state for nearly nine months. When host plants germinate the next spring in response to late autumn or winter rains, larvae break diapause and, if rains were sufficient, feed to maturity as solitary individuals (Murphy and White 1984). If rainfall was meager, it is believed many of the larvae feed for a few days and re-enter diapause (Singer and Parmesan 2010). Quino is likely to be found in barren spots surrounded by low-growing vegetation, especially their host plants and nectar sources. In Riverside County, the largest populations are found in coastal sage scrub habitat and in openings in redshank (*Adenostoma sparsifolium*) chaparral vegetation communities.

The distribution of Quino once spanned from the Santa Monica Mountains south to the northern parts of Baja California (USFWS 2003). However, nearly all of the butterfly's former range in California's native grasslands has been converted into a landscape dominated by human habitation or non-native plant species. Non-native plants, particularly Mediterranean grasses and forbs, provided better forage for livestock and rapidly outcompeted and replaced most native grassland vegetation (Seabloom et al. 2003). Thus, this butterfly's native grassland-associated larval host plants have been severely reduced in population size and are now restricted to a few localized areas. Preston et al. (2012) concluded that throughout the years, the distribution of Quino has shifted more inland toward the mountains. High amounts of grass can affect Quino habitat negatively as post-diapause Quino larvae tend to prefer areas with low grass coverage (Osborne et al. 2000), which allows them to have more solar exposure necessary for basking. If climate change causes increased drought or increased variability of rainfall patterns, as has been predicted for southern California (Seager et al. 2007; Diffenbaugh et al. 2008), the ties between pre-diapause larvae growth and host plant senescence may contribute to further declines in Quino populations.

The primary purpose of our Quino surveys is to monitor persistence of known populations and to ascertain the distribution of the species within apparently suitable habitat in the Conservation Area. Although we are not able to make an exhaustive search of this entire area, we endeavor to document the status of Quino at all of our established sites, and as time and personnel allow, expand our search to include other suitable or potentially suitable habitat. As a result of annual surveys through several years we have gained a better understanding of the overall distribution of Quino in our Conservation Area, as well as the relative stability of Quino populations (i.e., which locations continue to regularly support adult Quino and which locations had lower numbers of observed Quino). Since the development of a wildlife overcrossing in the Warm Springs Core Area in 2018 (specifically designed for the Quino checkerspot butterfly), we have surveyed the area on and around the overcrossing. In addition, in 2021 two wildlife cameras were installed at the overcrossing to further our search for Quino in this area. The use of

cameras has been shown to be effective in other studies when detecting lepidopteran species (McElveen and Meyer 2020).

Survey Goals and Objectives

1. Monitor Quino populations at sentinel sites.
    a. Determine the timing of the Quino flight season by surveying sentinel sites within 250 m x 250 m sampling station(s) to confirm presence/absence of Quino larvae and/or adults and their abundance.
    b. Track habitat conditions and species-specific resources on site.
2. Monitor Quino populations in areas with suitable habitat, with priority given to locations that were recently occupied.
    a. Conduct presence/absence surveys within 250 m × 250 m sampling stations at survey sites identified as having suitable habitat.
    b. Survey areas with known Quino populations to determine if sites are still occupied and the extent of occupation.
    c. Survey new areas with suitable habitat within designated critical habitat for Quino and surrounding areas.
    d. Map current observations to track distribution of Quino within the Conservation Area.

METHODS

Protocol Development

The Monitoring Program began developing a survey protocol in 2005 to determine the distribution of Quino across the Conservation Area. Survey goals in 2021 included monitoring the status of any locations with documented Quino populations within the last ten years. In addition to this goal, we monitored sites with historical Quino sightings and/or good potential for Quino occupancy in Core Areas, such as the Warm Springs Core Area. The collection of covariate data, such as temperature, wind speed, host plant distribution, and nectar plant presence during each survey aids our understanding of Quino resource selection. In 2021 our protocol also included the addition of two wildlife cameras on the Clinton Keith overcrossing located in the city of Murrieta within the Warm Spring Core Area.

Study Site Selection

Sentinel Sites

At the inception of our Quino monitoring effort in 2008, potential study sites were chosen using GIS layers of United States Fish and Wildlife (USFWS)-designated critical habitat for Quino and lands accessible to the Monitoring Program. Sentinel Site surveys occurred at sites which were geographically representative of the current distribution of Quino within the existing Conservation Area. We used ArcGIS (ESRI 2009) to delineate a 250 m x 250 m sampling station at each Sentinel Site. Sentinel Site locations were: Southwestern Riverside County Multi-Species Reserve (MSR) in the Johnson Ranch/Lake Skinner Core Area; Oak Mountain in the Oak Mountain Core Area; and a site near Tule Peak Road in the Silverado/Tule Peak Core Area (Figure 1). We assigned

one sampling station to all three Sentinel Sites: Oak Mountain, MSR, and Tule Peak Road.

Larvae and Adult Quino Survey Sites

In addition to the Sentinel Sites, surveys for adult Quino were conducted throughout six Core Areas: Warm Springs Creek Core Area, Sage Core Area, Johnson Ranch/Lake Skinner Core Area, Oak Mountain Core Area, Wilson Valley Core Area, and Silverado/Tule Peak Core Area (Figure 1). The Lake Mathews/Estelle Mountain/Harford Springs Core Area was not surveyed since Quino do not currently occupy this core. Using ArcGIS (ESRI 2009) we employed a grid of 250 m × 250 m sampling stations overlaid upon potentially suitable habitat in each Core Area. The number of sampling stations surveyed was variable depending on such factors as the degree of difficulty traversing the terrain, extent of suitable habitat, and the density of Quino in each sampling station.

Aside from the Core Areas surveyed, there were three non-core Satellite Occurrence Complexes (Dudek & Associates 2003) surveyed in 2021: San Bernardino National Forest, Cactus Valley, and Aguanga. As our understanding of Quino habitat suitability and knowledge of Quino occupancy evolves, and if we find Quino populations shift over time, more study areas may be added in subsequent years.

Adult Quino Camera Stations

The cameras were placed on a wildlife overcrossing located over Clinton Keith Road in the city of Murrieta within the Warm Springs Core Area. The Clinton Keith overcrossing is approximately 3.25km east of Interstate 215 and was completed in the Fall of 2018. The Clinton Keith overcrossing was originally built for the Quino checkerspot butterfly and has become an additional tool to detect butterfly species.

Survey Methods

Sentinel Site Visits

The primary purpose of Sentinel Site monitoring is to determine the timing of the Quino flight season at their most productive sites, which helps efficiently direct overall survey efforts. Secondary purposes are to track Quino habitat conditions on-site, including host plant distribution and abundance, and to document presence of Quino larvae, thus confirming Quino reproduction.

In 2021, surveys for Quino began in late January and continued through mid-June, and were timed to coincide with their four to six-week flight period. Flight start and end dates depend on the elevation of the site, temperature and rainfall. Sentinel Site visits commenced when spring conditions developed (i.e., sunny days with temperatures above 15°C). Surveyors visited each Sentinel Site to determine the commencement of the adult flight season. If Quino larvae were documented, adult Quino were typically observed on-site within two to four weeks.

Before departing to the field, surveyors uploaded waypoints into their handheld GPS units delineating the center of each sampling station at an assigned Sentinel Site. We

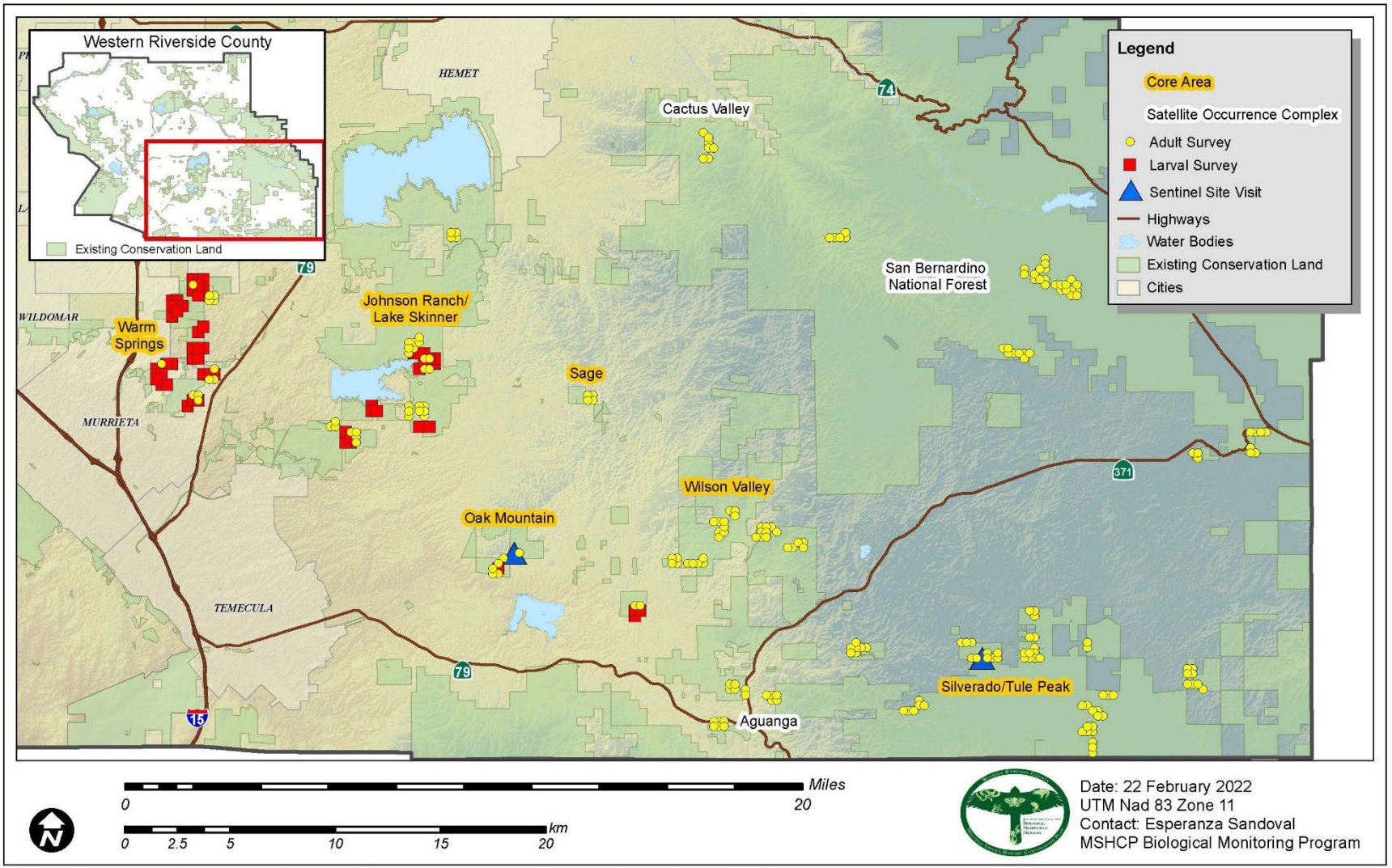

Figure 1. Quino checkerspot butterfly Sentinel Sites, larvae surveys, and adult Quino locations in 2021.

conducted surveys between the hours of 0930 and 1600 when temperatures in the shade at ground level were >15°C on a clear, sunny day or >21°C on an overcast or cloudy day, and with sustained wind speeds ≤ 24 km/h as measured 1.2–1.8 m above ground level (approximately chest height). Sustained wind was determined by averaging observed values over a 1-minute period. We did not conduct surveys when there was fog or precipitation.

Unless the above conditions precluded a Sentinel Site survey, the surveyor spent at least one hour searching the sampling station. Surveyors recorded number and behavior of Quino larvae and/or adults detected, available nectar sources, co-occurring butterflies, weather conditions, survey start and end time, and host plant status. For each species of host plant detected at any given survey, the number of individual plants was approximated and placed in one of three number ranges: 1-100, 101-1000, and more than 1000. Surveyors thoroughly covered each Sentinel Site using their knowledge of Quino ecology to maximize opportunities for detection. For instance, they spent time visiting hilltops and sandy washes, looking through patches of host plants, and scanning areas of flowering plants as part of the search effort.

Because Quino is a federally listed endangered species and because these Sentinel Sites represent some very good remaining habitat, surveyors were instructed to be extremely careful to avoid trampling larvae or host plants, disturbing cryptogamic soil crusts, or otherwise adversely impacting the resources at the site. We conducted Sentinel Site surveys until host plants had senesced or Quino were no longer detected. The survey methods are more completely described in the *Western Riverside County MSHCP Biological Monitoring Program 2021 Quino Checkerspot Butterfly Survey Protocol*.

Larvae Quino Surveys

The primary purpose of the larvae Quino surveys is to monitor the start of the Quino flight season. If the Quino larvae stage is detected on any given survey year, we would continue to survey the area weekly until adult Quino are detected. The secondary purpose is to collect sufficient environmental data that may contribute to a better understanding of any additional factors that influence the distribution, occurrence, and detectability of the species. Larvae Quino surveys commenced at the same time as Sentinel Site surveys, when spring conditions develop.

Before departing for the field, surveyors uploaded a series of waypoints into their handheld GPS units delineating the center of each sampling station at an assigned survey site. All other necessary survey conditions identified for Sentinel Site surveys (e.g., temperature, time of day, host plant status) applied to these surveys. Surveyors methodically searched for Quino larvae within sampling stations, giving preference to those portions that appeared more likely to support Quino larvae (e.g., occurrence of host plants and open areas). The surveyor spent at least one hour searching the sampling station. If Quino larvae were observed, we recorded a waypoint using a Garmin GPS unit, measured larvae length in millimeters (if possible), and documented behavior of Quino larvae (e.g., feeding, crawling) and substrate used (i.e., species of plant where the behavior was observed). With a few exceptions, most of the survey and scouting sites were visited only once or twice. Not all sampling stations at survey sites were visited due

to the large spatial extent of some sites or the lack of suitable habitat. Larvae Quino surveys concluded once the adult Quino is detected.

Adult Quino Surveys

The primary purpose of adult Quino surveys is to monitor persistence of known populations and to ascertain the distribution of the species within suitable habitat in the Conservation Area. The secondary purpose is to collect sufficient environmental data that may contribute to a better understanding of any additional factors that influence the distribution, occurrence, and detectability of the species.

Before departing for the field, surveyors uploaded a series of waypoints into their handheld GPS units delineating the center of each sampling station at an assigned survey site. Surveyors also took a map of the survey site to use in the field. Once assigned a given survey site by the Quino Survey Lead, surveyors were free to select sampling stations that they reasoned were more likely to be occupied by Quino based on a visual overview of habitat and previous knowledge of the area. All other necessary survey conditions identified for Sentinel Site surveys (e.g., temperature, time of day, host plant status) applied to these surveys. Surveyors methodically searched for adult Quino within sampling stations, giving preference to those portions that appeared more likely to support Quino (e.g., occurrence of host plants; suitable nectar sources; open areas, such as trails or washes; hilltops where Quino are known to congregate). These surveys were time-constrained to 45 minutes per sampling station to increase the amount of area surveyed per day. If Quino were observed, we recorded a waypoint using a Garmin GPS unit and documented Quino behavior (e.g., nectaring, ovipositing) and substrate used (i.e., species of plant where the behavior was observed). With a few exceptions, most of the survey and scouting sites were visited only once or twice. Not all sampling stations at survey sites were visited due to the large spatial extent of some sites or the lack of suitable habitat. Sampling stations were not resurveyed once we confirmed the presence of Quino.

Adult Quino Camera Stations

Camera traps have been previously effective in detecting butterflies and other insects (Edwards et al. 2015, McElveen and Meyer 2020). In 2021 we included cameras to increase our monitoring efforts at the Clinton Keith overcrossing in the City of Murrieta. The primary purpose of the camera stations is to detect Quino checkerspot butterflies using the Clinton Keith overcrossing in the Warm Spring Core Area. The second purpose is to collect any other butterfly species present in the area which can help us assess habitat suitability for butterflies.

Two Stealth cameras model DS4K were strapped to the fence bordering the Clinton Keith overcrossing. One is located on the West side and the other on the East side of the overcrossing. Both cameras were angled down towards nectaring sources in hopes of detecting butterflies nectaring. Uploaded photos from cameras were reviewed twice. The photos with no data were then deleted. The photos with data were saved and data was entered into an excel form, double checked, then entered into a database.

Training

In 2021 there were four surveyors who have passed the USFWS Quino identification exam. One surveyor has now 11 years of experience surveying for Quino, a second surveyor has now three years of experience, and the other two surveyors just completed their second season surveying for Quino in 2021. All surveyors have had training in house, in office, and in the field. Additionally, surveyors had demonstrable ability to identify the six plant species currently recognized as Quino host plants (USFWS 2003; *G. Pratt, personal communication*): California plantain *(Plantago erecta),* woolly plantain (*Plantago patagonica),* purple owl's clover *(Castilleja exserta),* Coulter's snapdragon (*Sairocarpus coulterianus),* Chinese houses *(Collinsia concolor),* and bristly bird's beak *(Cordylanthus rigidus)*. Also, two people began their Quino survey training in 2021 and shadowed trained personnel during multiple Quino surveys. Only fully trained, qualified Quino surveyors reviewed the photos taken at the Clinton Keith overcrossing in the Warm Spring Core Area.

Data Analysis

Data resulting from 2021 surveys were mapped and will be used to track distribution trends over time with the objective of understanding spatial and temporal fluctuations in the Quino population within the Conservation Area.

RESULTS

Overall, we surveyed from 28 January until 14 June, which includes Sentinel Site surveys, Quino surveys at the Core Areas, and Quino surveys at the Satellite Occurrence Complex Areas. We detected a total of 77 individual adult Quino (includes incidental observations; Figure 2) and surveyed a total of 367 sampling stations, which include every survey at the Sentinel Sites (n =23), every adult Quino survey at each sampling station (n = 284), and every repeated visit to the sampling stations where Quino was not detected on the first visit (n = 60). Out of the 367 sampling stations surveyed, Quino was detected during 11 of those surveys (3.0%).

The Quino flight season is determined by the first and last adult Quino observation detected in any given survey year. Between 2008 and 2021 adult Quino have been detected as early as 26 January (2011) and as late as 15 June (2010). The 2021 flight season was the second shortest flight season (shortest being in 2014) since the Monitoring Program began surveying for Quino (Figure 3). In 2021 the Quino flight season began with the first Quino sighting on 02 March at the Johnson Ranch/Lake Skinner Core Area, about one kilometer NW of the Multi-Species Reserve Sentinel Site (Figure 3; Figure 1). Adult Quino was not seen at the MSR Sentinel Site until two weeks later (Table 1). The Quino flight season ended with the sighting of two adult Quino on 08 April at the MSR Sentinel Site (Table 1, Figure 3). Our adult Quino observations occurred approximately between the hours of 0952 – 1412, with temperatures ranging between 17.9 – 31.1°C. We recorded wind speeds at the start and end of successful surveys to be between 0.6 – 3.9 km/h (average at start of survey = 2.25, average at end of survey= 2.1 km/h). In regards to cloud cover, including all 367 sampling stations surveyed, skies were clear from the start to the end of each survey in 95 of the sampling station (25.9%). Out of the 11 sampling stations surveyed where Quino was detected, eight sampling stations (72.7%) had clear skies at the start and end of each survey.

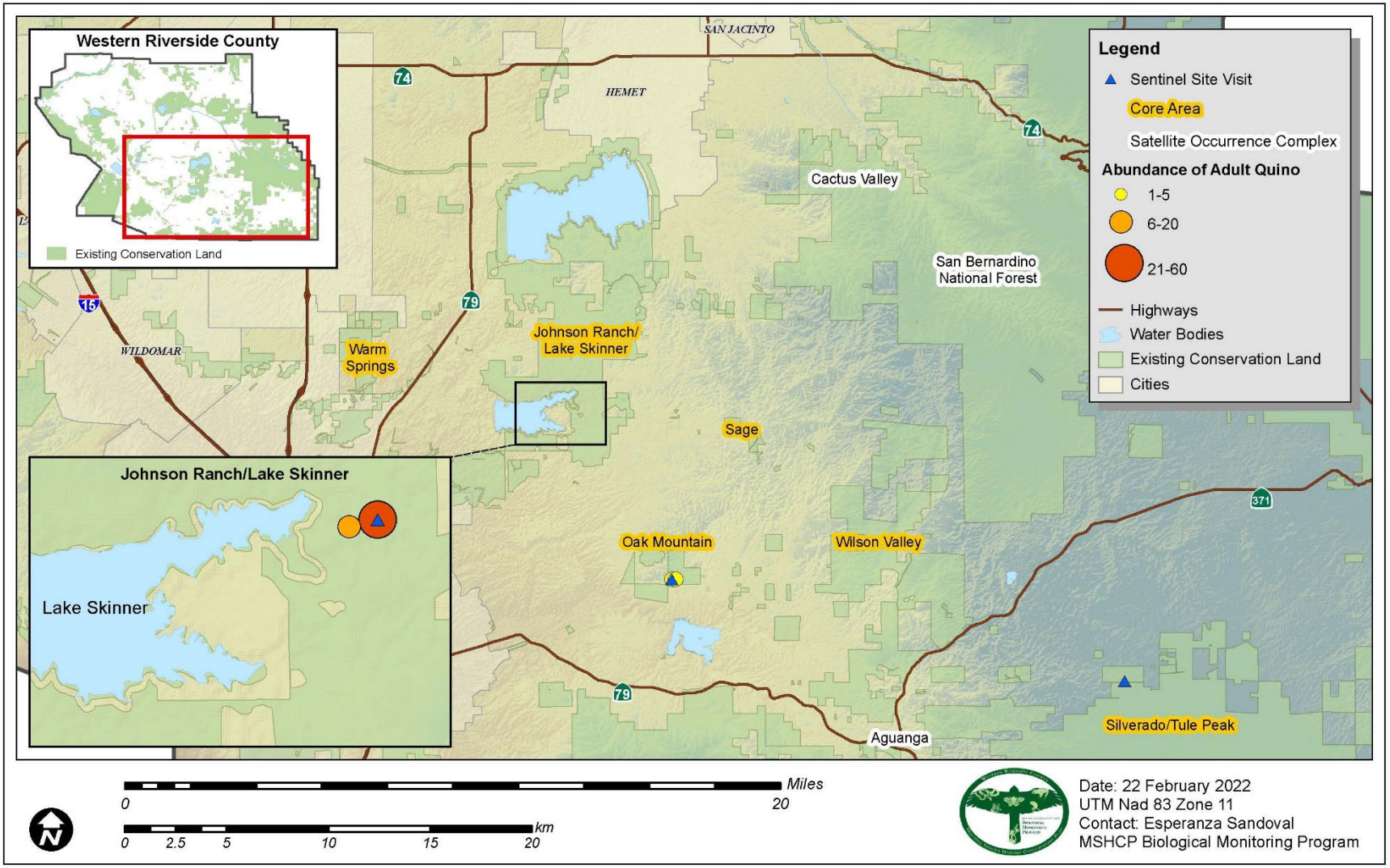

Figure 2. Quino checkerspot butterfly abundance by Survey and Sentinel Sites in 2021.

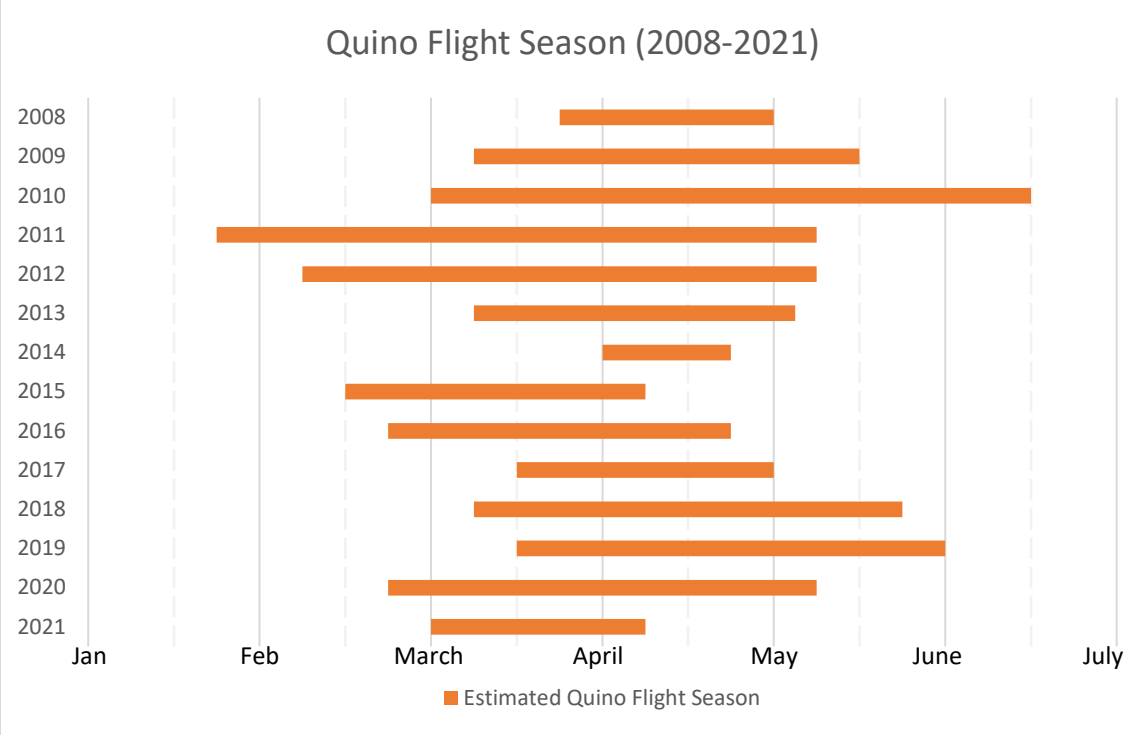

Figure 3. Quino checkerspot butterfly estimated observed flight season from 2008-2021. The number of surveyors and sampling station surveyed may have differed from year to year. Reports are available online: https://www.wrc-rca.org/species-survey

Sentinel Site Surveys

During the Sentinel Site surveys in 2021 Quino was detected at two out of the three Sentinel Sites, at MSR and Oak Mountain. The Silverado/Tule Peak Sentinel Site was surveyed on six separate occasions and despite our efforts, no Quino was detected. Of the 23 total visits conducted at our three Sentinel Sites, we detected adult Quino during six surveys (26.1%; Table 1).

Table 1. Larvae and adult Quino checkerspot butterflies observed during Sentinel Site visits during the 2021 flight season.

	Dates of Visits		Total # of Visits	Dates Quino Observed		Total # Quino Larvae Observed	Total # Quino Adults Observed
Sentinel Site	**First**	**Last**		**First**	**Last**		
Multi-Species Reserve	04 Feb	22 April	11	18 Mar	08 April	54	60
Oak Mountain	19 Mar	29 April	6	19 Mar	01 April	0	3*
Silverado/Tule Peak	20 April	27 May	6	-----	-----	0	0

*Includes 1 adult QCB incidental observation by Monitoring Program staff on 4 April 2021.

The Biological Monitoring Program visited the MSR Sentinel Site 11 times and observed 60 adult Quino during four visits (36.4%; Table 1) conducted on 18, 24 March, and 01, 08 April (*n* =29, 14, 15, and 2, respectively). In terms of adult Quino behavior, one was seen ovipositing on *Plantago erecta*, 17 were perched and/or basking, 18 were seen flying, 19 were exhibiting agonistic behavior, and five were seen nectaring. The butterflies were found nectaring on *Rhus ovata* (*n* =3), *Plagiobothrys* spp. (*n* =1), and *Gilia* spp. (*n* =1). A total of 54 Quino larvae were detected at MSR. Quino larvae were detected at three separate survey visits to the Sentinel Site conducted on 11, 17, and 25 February (*n* =22, 24, and 8, respectively). The majority of the Quino larvae were found feeding (*n* =29) on *Plantago erecta*, others were basking (*n* =19), and a few were crawling (*n* =6). There was an approximately three-week gap between the detection of the last Quino larva and the first Quino adult. The host plant *Plantago erecta* was abundant throughout the Sentinel Site during the larval and adult Quino surveys (Table 2).

The Biological Monitoring Program visited the Oak Mountain Sentinel Site six times in 2021 starting on 19 March and ending on 29 April. We observed 2 adult Quino during two visits (33%; Table 1) conducted on 19 March and 1 of April. On 4 April there was an incidental observation by Monitoring Program staff of one adult Quino at the Oak Mountain Sentinel Site. One adult Quino was seen basking, the other was seen flying, and the behavior of the incidental Quino observation was not recorded. This site still continues to have large patches of the host plant *Plantago erecta* throughout the site during the Quino flight season (Table 2). During the last visit on 29 April, half or more of the *Plantago erecta* present at the Oak Mountain Sentinel Site was senesced (personal observation). Aside from the host plant present there was also plenty of nectaring plants for the adult Quino to nectar. *Lepidium nitidum* (shining pepperweed), which is suspected to compete with other plants considered beneficial to Quino, was also present.

Table 2. Host plant species of the Quino checkerspot butterfly larva and adult Quino presence/absence observed during Sentinel Site visits in 2021.

Sentinel Site	Host Plant Species Detected	Quino Presence/Absence
Multi-Species Reserve	*Plantago erecta*	Present
Oak Mountain	*Plantago erecta*	Present
Silverado/Tule Peak	*Collinsia concolor* and *Sairocarpus coulterianus*	Absent

We visited the Sentinel Site in the Silverado/Tule Peak Core Area six times in 2021 and did not detect Quino in this area. The visits to this area began on 20 April and ended on 27 May. During our visits we detected a low number of host plants (Monitoring Program staff observation) throughout the Sentinel Site, *Collinsia concolor* and *Sairocarpus coulterianus* (Table 2). Several nectaring plant species were present throughout the Sentinel Site this year. We visited the MSR Sentinel Site 11 times in 2021 beginning 18 March and ending on 8 April. Overall, in 2021, nectaring Quino were only seen at one out of the three Sentinel Sites, at MSR (*n* = 5). Plants that we observed adult

Quino utilizing as nectar sources, in order of frequency of utilization, were: *Rhus ovata* (*n* = 3), *Gilia* spp. (*n* = 1), and *Plagiobothrys* spp (*n* = 1). Aside from Quino there were other co-occurring butterflies observed throughout the Sentinel Sites, including two species of checkerspots (Appendix A). The chalcedon checkerspot butterfly (*Euphydryas chalcedona chalcedona*) was observed at MSR and the Gabb's checkerspot (*Chlosyne gabii*) was observed at the Silverado/Tule Peak Sentinel Site. The common buckeye (*Junonia coenia*), whose larvae host plant include *Plantago* spp., was not detected during the 2021 Quino Sentinel Site surveys.

Larvae Quino Surveys

During the larvae Quino surveys the Biological Monitoring Program did not detect Quino larvae at any of the four Core Areas surveyed (Table 3) in 2021. Larvae surveys took place in four (Warm Springs Creek, Johnson Ranch/Lake Skinner, Oak Mountain, and Wilson Valley) out of the six Core Areas and none at the Satellite Occurrence Complex Areas.

Table 3. Larvae Quino occupancy at Core Areas in 2021.

Core Areas	No. of Visits	No. of Sampling Stations Surveyed	No. of Sampling Stations Occupied	No. of Adult Quino Present
Warm Springs	11	39	0	0
Sage	0	0	0	0
Johnson Ranch/Lake Skinner	10	20	0	0
Oak Mountain	1	1	0	0
Wilson Valley	1	3	0	0
Silverado/Tule Peak	0	0	0	0
Satellite Occurrence Complex Areas				
Cactus Valley	0	0	0	0
San Bernardino National Forest	0	0	0	0
Aguanga	0	0	0	0
Total	**23**	**63**	**0**	**0**

The Warm Springs Core Area was visited 11 times (*n*=39 sampling stations surveyed; Table 3) starting on 28 January to 26 February and no Quino larvae were detected. The host plant *Plantago erecta* was present in about 54% of the sampling stations surveyed.

The Johnson Ranch/Lake Skinner Core Area was visited ten times (*n*=20 sampling stations surveyed; Table 3) starting on 4 February to 2 March and no Quino larvae were detected. The host plant *Plantago erecta* was present in about 75% of the sampling stations surveyed.

The Oak Mountain Core Area was surveyed once (*n*=1 sampling station surveyed; Table 3) on 1 March and no Quino larvae was detected. The host plant *Plantago erecta* was present throughout the southern half of the sampling station surveyed.

We visited the Wilson Valley Core Area once (*n*=3 sampling station surveyed; Table 3) on 1 March and no Quino larvae was detected. The host plant *Plantago erecta* was present in two out of the three sampling station surveyed.

The host plant *Plantago erecta* was detected in all four of the Core Areas surveyed during Quino larvae surveys. No other co-occurring butterfly larvae species were detected during the Quino larvae survey, including the common buckeye (*Junonia coenia*), whose larvae feed on *Plantago* spp. At least five different species of adult butterflies were detected during these surveys but no checkerspot species were detected during the 2021 Quino larvae species.

Adult Quino Surveys

During the adult Quino surveys the Biological Monitoring Program observed Quino at two of the six Core Areas surveyed (Figure 2, Table 4) in 2021. No Quino detections occurred at the three Satellite Occurrence Complex Areas (Cactus Valley, San Bernardino National Forest, and Aguanga). Of the 14 total adult Quino individuals observed during the 2021 season, the largest number (*n* = 13) were found at the Johnson Ranch/Lake Skinner Core Area followed by the incidental observation at the Oak Mountain Core Area (*n* = 1; Table 4). Quino larvae were not detected at any of the Core Areas or Satellite Occurrence Complex.

Table 4. Adult Quino occupancy at Core Areas in 2021.

Core Areas	No. of Visits	No. of Sampling Stations Surveyed	No. of Sampling Stations Occupied	No. of Adult Quino Present
Warm Springs	7	26	0	0
Sage	2	4	0	0
Johnson Ranch/Lake Skinner	12	33	5	13
Oak Mountain	5	7	0	1*
Wilson Valley	8	36	0	0
Silverado/Tule Peak	23	66	0	0
Satellite Occurrence Complex Areas				
Cactus Valley	3	7	0	0
San Bernardino National Forest	12	45	0	0
Aguanga	3	17	0	0
Total	**98**	**284**	**5**	**14**

*Includes 1 adult QCB incidental observation by Monitoring Program staff on 19 March 2021.

We visited the Warm Springs Core Area seven times (*n* = 26 sampling stations surveyed; Table 4) starting on 5 March to 6 April and no Quino were detected. Two host

plants species were detected in the Warm Spring Core (Table 5). Robust patches of the host plant *Plantago erecta* were present in approximately 50% of the sampling stations surveyed and *Castilleja exserta* was only detected in one area surveyed, the Clinton Keith overcrossing (Biological Monitoring Program 2021) located over Clinton Keith Rd. in Murrieta, CA (about 3km East of Interstate 215).

We visited the Sage Core Area twice (n = 4 sampling stations surveyed; Table 4) on 26 and 31 March and no adult Quino were detected. The host plant *Plantago erecta* continues to be present at this location and it was detected on all the 4 sampling stations surveyed (Table 5). Native wildflowers were represented throughout the sampling stations surveyed, which can provide nectaring sources for butterfly species, but non-native grasses were also detected which is not ideal for Quino habitat.

The Johnson Ranch/Lake Skinner Core was visited 12 times (n = 33 sampling stations surveyed; Table 4) starting on 5 March to 22 April. A total of 13 adult Quino were detected in five out of the 33 sampling stations surveyed (15.2%; Table 4) during four visits to the Johnson Ranch/Lake Skinner Core Area. One adult Quino was identified on 2 March, five on 18 March, six on 19 March, and one on 24 March. Observed Quino behaviors included, three that were flying and ten that were perched or basking on three different plant species (*Plantago erecta, Artemisia californica,* and *Eriogonum fasciculatum*). The host plant *Plantago erecta* was present in about 70% of sampling stations surveyed and *Castilleja exserta* was present in about 12% of the sampling stations surveyed (Table 5).

The Biological Monitoring Program paid five visits (n = 7 sampling stations surveyed; Table 4) to the Oak Mountain Core Area starting on 19 March to 15 April with no Quino detected during the surveys, but an incidental observation of one adult Quino was reported on 19 March by Monitoring Program staff (Table 4). The incidental was made after the completion of the surveys for that day. The adult Quino was first detected as it flew across the dirt road then landed and began nectaring on a *Ceanothus* spp. Large patches of *Plantago erecta* were detected in about 71% of the sampling stations surveyed. Other nectaring sources were present in all seven sampling stations surveyed (Table 5).

We visited the Wilson Valley Core Area eight times (n = 36 sampling stations surveyed; Table 4) in 2021 starting on 22 March to 27 April with no Quino detections. The host plant species *Plantago erecta* was detected in 5.6% of the sampling stations surveyed and *Collinsia concolor* was detected at approximately 14% of the sampling stations surveyed (Table 5). The host plant *Castilleja exserta* was only found on the Center for Natural Lands Management (CNLM)-managed land North of the Wilson Valley Road and Thomas Road intersection.

The Silverado/Tule Peak Core Area was visited 23 times (n = 66 sampling stations surveyed; Table 4) starting on 9 April to 27 May with no Quino detections. The areas that were surveyed include sampling stations located near Beauty Mountain, Anza-Borrego, Misty Meadows Drive, Barbara Trail, Tule Peak Road, and Bowers Road. Out of the 66 sampling stations surveyed, 24 of them were at the latter two locations as these sites were occupied during surveys conducted in collaboration with USFWS range-wide monitoring efforts in 2008. The host plant species *Collinsia concolor* was present in

approximately 20% of the sampling stations surveyed, *Sairocarpus coulterianus* was present in 14%, and *Castilleja exserta* was present in only one sampling station surveyed in the Anza-Borrego Core Area (Table 5). For multiple years the Silverado/Tule Peak Core Area had been a reliable location for the Biological Monitoring Program to detect adult Quino (Appendix B), but proved not to be the case in 2021.

The Cactus Valley Satellite Occurrence Complex (Brown Canyon site) was visited three times (n = 7 sampling stations surveyed; Table 4) starting on 30 March to 16 April with no Quino detections. Quino host plants were present in all seven of the sampling stations surveyed. The host plant species *Plantago erecta* and *Castilleja exserta* were the most widespread host plant species and both were present in approximately 71% of the sampling stations surveyed. *Collinsia concolor* and *Plantago patagonica* were only found in one sampling station each (14% of sampling stations surveyed). In 2021 four out of the six known host plants were detected in the Cactus Valley Satellite Occurrence Complex.

The San Bernardino National Forest Satellite Occurrence Complex was visited 12 times (n = 45 sampling stations surveyed; Table 4) starting on 2 April to 14 June with no Quino detections. The areas that were surveyed include sampling stations located at our Horse Creek site (ranges in elevation between 820-900 meters), the Hog Lake Truck Trail site (ranges in elevation between 1220-1400 meters), and the higher elevation SBNF site by Rouse Hill Road (ranges in elevation between 1600-1900). We visited the higher elevation area by Rouse Hill Road seven times in light of publications stating Quino are believed to be colonizing higher elevation sites (Parmesan 1996), but were unable to detect any Quino. We detected one adult Quino at an approximate elevation of 1831 meters at the SBNF site in 2017, but none since. The Hog Lake Truck trail site and the Rouse Hill Road site had detections of two host plant species. *Collinsia concolor* was present in approximately 24% sampling stations surveyed and *Sairocarpus coulterianus* was present in approximately 9% of the sampling stations surveyed (Table 5).

This has been the second consecutive year the Biological Monitoring Program has surveyed for Quino at the Aguanga Satellite Occurrence Complex. In 2021 we visited this core three times (n = 17 sampling stations surveyed; Table 4) starting on 17 March to 31 March with no Quino detections. No host plant species have been detected in this area (Table 5).

The two Quino-occupied Core Areas (Johnson Ranch/Lake Skinner and Oak Mountain) had *Plantago erecta* as the major Quino host plant, as did the three unoccupied Cores (Warm Springs Creek, Sage, and Wilson Valley). The host plants *Sairocarpus coulterianus* and *Collinsia concolor*, were present at the remaining occupied Core Area (Silverado/Tule Peak). We found five host plant species at two (Cactus Valley and SBNF) out of the three Satellite Occurrence Complex, *Plantago erecta, Collinsia concolor, Castilleja exserta, Plantago patagonica*, and *Sairocarpus coulterianus* (Table 5). *Plantago erecta* was the major host plant for the Cactus Valley Satellite Occurrence Complex and *Collinsia concolor* was the major host plant for the SBNF Satellite Occurrence Complex. *Cordylanthus rigidus* was not encountered during survey efforts throughout the whole 2021 Quino season.

Table 5. Host plant species of the Quino checkerspot butterfly larva and adult Quino presence/absence observed during adult Quino surveys in 2021.

Core Areas	Host Plant Species Detected	Quino Presence/Absence
Warm Springs Creek	*Plantago erecta* and *Castilleja exserta*	Absent
Sage	*Plantago erecta*	Absent
Johnson Ranch/Lake Skinner	*Plantago erecta* and *Castilleja exserta*	Present
Oak Mountain	*Plantago erecta*	Present
Wilson Valley	*Plantago erecta, Castilleja exserta*, and *Collinsia concolor*	Absent
Silverado/Tule Peak	*Castilleja exserta, Collinsia concolor*, and *Sairocarpus coulterianus*	Absent
Satellite Occurrence Complex Areas		
Cactus Valley	*Plantago erecta, Plantago patagonica, Castilleja exserta*, and *Collinsia concolor*	Absent
San Bernardino National Forest	*Collinsia concolor* and *Sairocarpus coulterianus*	Absent
Aguanga	None detected	Absent

Of the 14 adult Quino observations in 2021, one detection was made incidentally and 13 were observed during surveys across all sampling stations (n = 284, Table 4) during the adult Quino surveys (includes Core Areas and the three Satellite Occurrence Complex). The incidental observation at the Oak Mountain Core Area was the only adult Quino seen nectaring in 2021 and chose to nectar on *Ceanothus* spp. Aside from the Quino checkerspot butterfly, there were several other co-occurring butterflies observed throughout the Core Areas, including two species of checkerspot butterflies (Appendix A). The chalcedon checkerspot butterfly (*Euphydryas chalcedona chalcedona*) was observed at the Johnson Rank/Lake Skinner Core Area, the Wilson Valley Core Area, the Oak Mountain Core Area, the Silverado/Tule Peak Core Area, the Warm Springs Core Area, and the SBNF and Aguanga Satellite Occurrence Complex. The Gabb's checkerspot (*Chlosyne gabbii*) was observed at the Silverado/Tule Peak Core and the SBNF Satellite Occurrence Complex. The common buckeye (*Junonia coenia*), whose larvae feed on *Plantago* spp., was not detected throughout the 2021 Quino surveys.

Adult Quino Camera Stations

The camera stations at the Clinton Keith overcrossing in the Warm Springs Core Area did not have any Quino detections. The Stealth cameras were active from 23 March

until 1 July. Other co-occurring butterfly species were detected by the Stealth camera including the chalcedon checkerspot butterfly (*Euphydryas chalcedona chalcedona*).

DISCUSSION

Although the survey effort in 2021 (includes Sentinel Site surveys, adult surveys, and scouting surveys) increased, the number of Quino detected was lower than the previous two years. In the four most recent years, we observed a total of 77 adult Quino during 367 sampling stations surveyed (includes Sentinel Sites, larval and adult survey sites, repeat visits, and incidental observations) in 2021 (mean = 0.21 Quino per visit), we observed a total of 441 adult Quino during 239 sampling stations surveyed in 2020 (mean = 1.85 Quino per visit), 199 adult Quino during 168 sampling stations surveyed in 2019 (mean = 1.18 Quino per visit), and 84 adult Quino during 148 Sampling stations surveyed in 2018 (mean = 0.57 Quino per visit). The variation in the number of Quino throughout the years could be due to the fluctuation in the number of qualified surveyors in relation to the area being surveyed, but it doesn't explain the lower observation numbers in 2021. In 2019 and 2018 there were 1-2 qualified Quino surveyors available throughout the season, in 2020 we had 2-4 qualified Quino surveyors, and in 2021 we had four reliable and trained surveyors throughout the Quino season. With the increase in surveyors, we were able to cover more area, which led to a higher number of sampling stations surveyed. The number of sampling stations surveyed per site varied due to the amount of accessible conserved land, the suitability of habitat within sampling stations, and the number of survey days available. No Quino were observed at seven of the nine Core Areas and Satellite Occurrence Complexes in 2021. Even though more area was covered in 2021 our Quino detections of 77 adult Quino were the lowest number observed during the past four years, only coming close to 2018 when we detected 84 adult Quino and surveyed about 60% less sampling stations.

A possible reason for the lower numbers of Quino detections in 2021 could be due to lower annual rainfall. In the past five years the lowest estimated annual rainfall occurred in 2018 and in 2021 (Appendix B). Those two years with the lower rainfall numbers seem to coincide with the lower Quino totals. Lower amount of rain can cause stress on the annual Quino host plants. The host plants can instead save their seed bank for the following year if conditions are not favorable which can lead the post-diapausal Quino larvae with a limited food source that may or may not be enough to acquire enough energy to enter diapause (Ehrlich et al. 1980). In order for the post-diapausal Quino larvae to have enough nourishment from the lower number of host plants present they would need to decimate most of the host plant population. This can be problematic once the larvae pupate into adults and the female Quino will attempt to locate host plant to oviposit. In this case adult Quino have been seen dispersing themselves greater distances to find suitable host plants (Murphy and White 1984). One Core Area surveyed in 2021 that clearly had a decrease in host plant was the Silverado/Tule Peak Core Area (Sandoval, Esperanza, SAWA Biologist II, 2021, personal observation).

Over the past 14 flight seasons (2008-2021), we have not detected Quino in the Warm Springs Creek Core Area (Appendix B) despite the presence of robust patches of *Plantago erecta* in many areas, and large expanses of suitable habitat. Within this Core Area, we have surveyed the Anheuser-Busch site, Phase 1-5 and Phase 8 eight years

(2013, 2015, 2016, 2017, 2018, 2019, 2020, 2021) with no success despite the fact that it contains suitable habitat, including abundant stands of Quino host plants, but it also has seen an increase in non-native grasses covering up the nectaring sources. With the addition of the Clinton Keith overcrossing (completed Fall 2018) in the Anheuser-Busch site Phase 1 we have been surveying the overcrossing and surrounding sampling stations since 2019. It currently has *Eriogonum fasciculatum* and *Acmispon glabrus* growing on the overpass as well as host plants such as *Plantago erecta* and *Castilleja exserta*. Once *Eriogonum fasciculatum* matures, the overcrossing will be more promising for Quino. If Quino were to re-colonize this area at some time in the future, or if Quino were to be translocated here, successful establishment may likely occur.

Our only survey site in the Sage Core Area, Magee Hills, is rather isolated from other occupied areas. The most proximal occupied site is 7.5 km from the Magee Hills site. In 2021 we did not detect Quino in this area, which could be due to how the survey site visits were arranged. Due to the truncated timing of the 2021 Quino flight season, not all sites were able to be surveyed at the ideal time for detection, such as Magee Hills, where it's possible our surveys took place before the Quino flight season or after the flight season had ended. The encroaching non-native grasses that are taking over the open areas where Quino bask and mate could be another reason why Quino were not detected in this area. Our visits to Magee Hills were few and not ideal due to weather. Our first visit was on 26 March and the second and last visit was on 31 March. The second visit was cut short when weather was no longer suitable for butterflies. There is a possibility that we missed the Quino flight season in this area completely by not visiting sooner in the season. During the two visits *Plantago erecta* was detected in several sampling stations surveyed and was recorded as flowering at the time of the surveys. There were open areas filled with nectaring sources but the open areas are becoming smaller due to the non-native grasses and Sahara mustard (*Brassica tournefortii*). In the absence of management to reduce the cover of these invasive species, Quino may become extirpated from this area, especially if their basking and mating sites disappear with the increase in non-native vegetation. Over 14 years surveying this site, we have been successful at detecting a small but persistent population of Quino approximately 43% of the time (Appendix B).

In 2021, in the Johnson Ranch/Lake Skinner Core Area, the most productive site was the Sentinel Site (Figure 2) followed by the sampling station southwest of it. This year there were large patches of *Plantago erecta* found throughout the sampling stations surveyed. The sampling station surveyed adjacent to the Multi-Species Reserve Sentinel Site, had a few plants of *Castilleja exserta* and large, open areas. Several areas around these sampling stations are continuing to see non-native grass growth and habitat suitability has been slowly decreasing over the past decade. The Johnson Ranch/Lake Skinner Core Area continues to be one of the best areas to find Quino in Western Riverside County.

In 2021, Oak Mountain's Quino detection numbers were low. A total of four adult Quino were detected, three at the Sentinel Site (includes two Quino detected during sentinel site visits and an incidental observation; Table 1) and one incidental observation detected after completing an adult Quino survey on an adjacent sampling station to the sentinel site (Table 4). Total adult Quino numbers, includes detections at Sentinel Sites

and during adult survey sites, seem to be decreasing in this area since 2017 (n = 85), which was a year with greater rainfall compared to subsequent years (Appendix B). As mentioned before, rainfall can be a big factor in host plant availability for Quino larvae (Ehrlich et al. 1980) but this area does continue to house large patches of *Plantago erecta*. In 2021, despite having large patches of *Plantago erecta* our Quino totals were low. These low observations of Quino could have been due to timing of the visits. The first visit took place on 1 March and surveys were done at a lower elevation area near Vail Lake but we didn't get to the Sentinel Site until 19 March when we detected the first Quino at Oak Mountain. It's possible we surveyed late in the flight season and missed the peak season of adult Quino but we did confirm Quino presence.

We did not find Quino in the Wilson Valley Core Area in 2021. The last Quino detected in the Wilson Valley Core Area was in 2013 (Appendix B). We were able to survey the sampling stations where Quino were last documented but we cannot claim to have thoroughly searched this entire area, which is extensive. We found small amounts of *Plantago erecta* and *Collinsia concolor* but also non-native vegetation encroaching on some open areas. We did expand our search in other areas of the Wilson Valley Core, including an area managed by CNLM, located north of the Wilson Valley Road and Thomas Road intersection, and found potential habitat. There could be a complexity of reasons as to why Quino might not be present in some areas such as urbanization, climate change, and a decrease in wildflower production (Preston et al., 2012). As the habitat keeps changing, we want to make sure we survey our historical sites as well as surrounding areas and continue expanding the search for Quino in the Wilson Valley Core Area.

The Silverado/Tule Peak Core Area has proven to be a reliable location for Quino presence for 13 consecutive years (2008-2020; Appendix B). In 2021, despite our efforts, we did not detect any Quino in this Core Area. We initially intended to visit this Core Area in late March, similar to surveys conducted in 2020, but in 2021 cooler temperatures in late March delayed the first visit to the Silverado/Tule Peak Core Area until 9 April. The late start in surveying this area and/or the lower number of host plants observed during surveys could explain the lack of Quino observations in the Silverado/Tule Peak Core Area. In previous years the Biological Monitoring Program have observed areas along Tule Peak Road, which have been the most productive in the past, with patches of *Castilleja exserta* and *Collinsia concolor*. In 2021 the only area in the Silverado/Tule Peak Core with *Castilleja exserta* observations was in Anza-Borrego. It appears the Sentinel Site within this Core had a lower number of host plants compared to last year (personal observation). In 2020, we documented *Collinsia concolor* abundance in the range of 101-1000 individual plants at the Sentinel Site, whereas in 2021 we documented 1-100 individuals. Presence of *Castilleja exserta* was recorded at the Sentinel Site in 2020 but none were observed in 2021. A Quino checkerspot butterfly requires the presence of ample amounts of host plants in order to survive into adulthood (Pratt and Pierce, 2010). We were able to expand our search in the Silverado/Tule Peak Core Area and surveyed a new area by Barbara Trail and it would be worth to continue to survey this area in the future. In the Silverado/Tule Peak Core Area we were able to visit Beauty Mountain four times throughout the month of May but it is a large area to cover. More

Quino may be found in the Beauty Mountain area if we expand our survey effort during future site visits.

We did not detect Quino in Brown Canyon in the Cactus Valley Satellite Occurrence Complex in 2021. Quino had not been detected in this area since 2010 despite several survey attempts (Appendix B). In 2018 we were able to survey new sampling stations in the Brown Canyon area with suitable habitat and were successful at detecting Quino (Appendix B). We returned to those sampling station in 2020 twice and now in 2021 we visited on 30 March, 9 and 16 April. The Biological Monitoring Program detected large patches *of Plantago erecta* in at least one sampling station but its presence was noted in other sampling stations as well. The host plants *Castilleja exserta, Plantago patagonica, and Collinsia concolor* were also present throughout the area surveyed but no Quino was detected. There is a possibility that we missed Quino in this area completely due to timing of the visits. The area was surveyed for three consecutive weeks and on the first visit green and flowering *Plantago erecta* was detected and continued to flower for the three weeks we surveyed. If time and personnel allowed it, we could have continued to survey this area and cover more sampling stations. It seems likely that if adult Quino would have been present, we would have detected it due to the stage of the host plant, but we cannot claim to have thoroughly searched this area. The presence of non-native grasses is dominant in some areas but there is still good suitable habitat. We plan to continue to survey the Cactus Valley Satellite Occurrence Complex in this area to determine the extent of Quino distribution.

The Aguanga Satellite Occurrence Complex was surveyed for the second consecutive year. We were only able to survey once west of the state route 371, once east of the state route 371, and once south of the state route 79. There is a possibility Quino was not detected in this area due to the low number of visits dedicated to this area. The Biological Monitoring Program hopes to continue surveying in this area and survey additional sampling stations.

According to the Species Account (Dudek & Associates 2003), Quino have been extirpated from the Lake Mathews/Estelle Mountain/Harford Springs Core Area. Quino were historically abundant in the Harford Springs subunit, but were last documented in Harford Springs Park in 1998 (USFWS 2003, Krofta and Anderson 2002) and local experts noted the abrupt decline of Quino colonies in the Gavilan Hills and near Lake Mathews during the early 1980's (Mattoni et a1. 1997). Surveys were conducted over eight years by the Biological Monitoring Program biologists with no success, leading to termination of surveys in this Core Area beginning in 2012 (Appendix B). In 2020 we decided to visit the northern area of Hartford Springs Park, mainly for training purposes, and did not detect any Quino. There was plenty of non-native grass throughout the area surveyed and did not detect any host plants. This area was not surveyed in 2021.

In 2021, we were able to detect two stages of the Quino life cycle (larval and adult form) in one out of the two Core Areas with Quino presence, the Johnson Ranch/ Lake Skinner Core Area. Timing of the surveys could have been a factor as to why Quino larvae were detected in one Core Area and not the other. We began surveying the MSR area and the Johnson Ranch/Lake Skinner Core Area in early February and detected young *Plantago erecta* throughout the sampling stations surveyed. On the second, third,

and fourth visit (11, 17, 25 February, respectively), post-diapausal Quino larvae were detected. Due to unsuitable butterfly weather (cool temperatures and rain) there were no surveys between 9 March through 17 March. Quino surveys resumed 18 March, which would have been plenty of time for the Quino larvae to pupate and emerge into adults, and we detected adult Quino at the MSR Sentinel Site (Mattoni et al. 1997). In Oak Mountain, we detected adult Quino during the first visit to the Sentinel Site on 19 March, so we most likely missed the window for detecting Quino post-diapause larvae. We failed to detect Quino larvae and adult presence in all other sites surveyed during the 2021 survey effort, despite the presence of host plants and nectaring sources in some areas.

Differences in flight season have been evident throughout the years (Figure 3). Aside from environmental factors some of these differences could be due to adjustments in survey effort and survey methods throughout the years. In 2010-2012, the flight season extended over a 12- to 14-week time period. Over the past three years the Quino flight season has been approximately eleven and a half-week in length. In 2021 it has shortened to about five and a half-week flight season, with the first Quino adult observed on 2 March and the last observation occurred on 8 April (Figure 3).

Distribution of Quino in 2021 was within the southern half of the Plan Area, bounded by the SBNF Satellite Occurrence Complex area to the east, Silverado/Tule Peak Core Area to the southeast, and the MSR sites and Oak Mountain Sentinel Site to the west. The Quino sites in the western portion of the Plan Area are lower in elevation (400 m – 850 m) than the southeastern and eastern sites (925 m – 2000 m). Of the sites surveyed in 2021, Anheuser-Busch and Winchester 700A (in the Warm Springs Creek Core Area) were the lowest elevation sites (approximately 400 m) and the higher elevation SBNF site by Rouse Hill Road (in the SBNF Satellite Occurrence Complex) was the highest (approximately 1900 m). If Quino shift to higher elevation habitat, this area could support new Quino populations in the future. Two species of host plants (*Collinsia concolor* and *Sairocarpus coulterianus,* Table 5) were detected in the Rouse Hill Road area in 2021, *Collinsia concolor* being the most abundant of the two and present in 10 out of the 22 sampling stations surveyed. Quino have been documented in this area at approximately 1707 meters in elevation (*James Gannon, Bureau of Land Management, personal communication*) in 2011 and as high as 1855 meters in elevation during 2017 adult Quino survey. This became the highest elevation Quino sighting ever recorded, and is the highest elevation site documented by the Monitoring Program.

Of the sites occupied by Quino in 2021, the lowest in elevation were the sampling stations at Johnson Ranch/Lake Skinner Core Area (approximately 475 m) and the highest elevation occupied site was Oak Mountain Core Area (approximately 800 m). We did not detect any Quino at the Rouse Hill Road site within the SBNF Satellite Occurrence Complex in 2021, but we will continue to document the elevations at which Quino are detected within the Plan Area to track distributional shifts over time, especially in light of a hypothesis that Parmesan (1996) suggests Quino will shift north and to higher elevations due to climate change.

Changes in the protocol could have affected adult Quino detections at the Oak Mountain Sentinel Site. In 2018 rather than treating the whole Oak Mountain Core Area as a Sentinel Site (as was done prior to 2018), we constrained our efforts to two sampling

stations where Sentinel Site surveys were conducted. This modification to the protocol could have affected the number of adult Quino detected since the area considered a Sentinel Site decreased. In 2019, we constrained our efforts further by designating one sampling station as our Oak Mountain Sentinel Site, which can impact adult Quino detections once more. All three of our Sentinel Sites are now defined by one sampling station each. In 2021 we detected more adult Quino in the Multi-Species Reserve Sentinel Site (n = 60) than we did in the Oak Mountain Sentinel Site (n = 3, Table 1). Another difference between survey years was the amount of *Lepidium nitidum* growing onsite. Although *Lepidium nitidum* is native to California, in 2017 it buried host plants in some areas of Oak Mountain and decreased the amount of open ground. In 2018, the cover of *Lepidium nitidum* decreased, during the lower annual precipitation compared to 2017 (Appendix B). In 2019, 2020, and 2021 *Lepidium nitidum* was still present in Oak Mountain.

Recommendations

Future Surveys: Both the extent of occupied area within each survey site and the number of occupied sites across the Conservation Area vary from year to year. Mapping the extent of occupied area within each survey site is more time-consuming, while determining the distribution of Quino across the Conservation Area as a whole is the more relevant MSHCP monitoring goal, and therefore we will prioritize monitoring at this scale. We should continue to monitor recently occupied sites and areas with apparently suitable habitat, or areas that are adjacent to known occupied habitat. If data suggests that Quino meta-populations and suitable habitat are shifting, Sentinel Site locations will need to shift accordingly for future surveys.

We have not detected Quino in the Warm Springs Creek Core Area over the past 13 years of survey efforts. The wildlife bridge (overcrossing) that spans Clinton Keith Road may facilitate Quino movement between formerly fragmented habitat in this Core Area. The Biological Monitoring Program has proposed a plan to conduct five years (2020-2024) of surveys at sampling stations near the overcrossing to detect Quino occupancy and document habitat attributes. In 2021, we detected host plant species, such as *Plantago erecta*, and shrub species, such as *Acmispon glabrus* and *Eriogonum fasciculatum* on the overcrossing. *Eriogonum fasciculatum* can be a source of shelter (as mentioned before, Quino larvae have been seen diapausing at the base of this shrub) and might play an important role in habitat restoration for Quino that reside in dry areas (Pratt and Emmel, 2010), such as the Warm Springs Core Area. The presence of shrubs and host plants on the overcrossing is a step in the right direction but we recommend that survey efforts be expanded to other areas with suitable habitat within this core on both sides of the overcrossing and that encroaching non-native grasses to be removed.

In 2021 we continued to expand our scouting and surveying efforts to include the Aguanga Satellite Occurrence Complex and the Wilson Valley Core Area. We were only able to visit the Aguanga area three times covering three different locations in this Satellite Occurrence Complex. Quino continues to occupy the Wilson Valley Core Area in small numbers, but our sites are no longer highly suitable. Because Quino occur as meta-populations, it is very possible we are missing currently occupied habitats when we survey at only historically occupied locations; exploring other potential areas may be

fruitful. In 2019, we expanded our surveys to a new area of Wilson Valley, adjacent to Wilson Valley Road, and those areas were again surveyed in 2020 and 2021. Suitable habitat was detected in these new sampling stations in Wilson Valley so it would be appropriate to survey those areas again and others during the Quino flight season. Habitat adjacent to Wilson Valley Road has been quite reliable for Quino sightings in the past. We intend on surveying both the Aguanga and Wilson Valley Core Area more thoroughly during future survey efforts.

We believe it is important to survey areas at higher elevations, such as Rouse Hill Road (ranges in elevation between 1600-1900m), as these may be occupied by Quino populations no longer occupying habitats at lower elevations. Where Quino host plant locations are known, especially in the higher elevations, it may be useful to scout these areas for Quino occupancy. This could serve to increase our knowledge of Quino distribution. Also, we need to remember that in order for Quino successfully maintain occupancy of lands, there needs to be connectivity to facilitate their movement between populations, and not blocked by urban environments (Parmesan et al., 2014). Additionally, we would like to increase our survey efforts near the currently occupied Beauty Mountain site in the Silverado/Tule Peak Core Area, which is at approximately 1400 m in elevation.

It may be productive to scout more area of Bautista Canyon, including our original Horse Creek site in the SBNF Satellite Occurrence Complex, which was surveyed from 2006-2010 and was found to be occupied by a small number of Quino. We surveyed an area north of the Horse Creek drainage where the Biological Monitoring Program discovered a reliable location for Quino in 2012. In 2018-2021 we surveyed a wash just north of Horse Creek and found Quino in 2020, but none in 2021. In prior years, we didn't have the resources to include this site and more recently a fence blocking entrance to the creek caused uncertainty in authorized access to this area. Our present site is close enough in proximity to the original known location to be part of the same meta-population, but we have not surveyed the original site since 2010. Once access to Horse Creek has been resolved, we hope to continue surveys in our original Horse Creek site. It would be interesting to know the full extent of this Quino population and other populations in Bautista Canyon.

We also should focus our attention at Magee Hills in the Sage Core Area since we detect Quino intermittently in this area. The last year Quino was detected at Magee Hills was in 2018. Too much growth of non-native grasses and other invasive plant species is changing the habitat in this site. Management is necessary for Quino to continue the use of Magee Hills. We intend to continue surveys in this area during future survey efforts. Lastly, in the Oak Mountain Core Area, the Biological Monitoring Program was able to survey down-slope towards Vail Lake in 2021 (twice in March). We do know there are large patches of *Plantago erecta* in this area and that Quino occupied these areas as recently as 2009. Even though no Quino detections were made in 2021, it was a drought year and it would be appropriate to re-survey this area to update our current knowledge of Quino distribution.

Conservation and Management: It is likely there are important differences in vegetative and other habitat conditions at occupied areas compared to unoccupied areas.

It is also possible that some areas with habitats that are entirely suitable for Quino are not occupied due to barriers to dispersal, development projects, present drought conditions, or other factors preventing Quino from occupying the site. More research is needed to determine if the present restricted distribution of Quino is a condition that will persist or, if or when the continuing drought or other unfavorable conditions are relieved, Quino will re-occupy other areas with suitable habitat.

The Oak Mountain Core Area is one of the best remaining areas for Quino occupancy. As Oak Mountain continues to be developed, the remaining open land is very crucial to Quino persistence. The RCA is in the process of reaching out to landowners that support high quality Quino habitat to discuss possible acquisition(s). The land on top of Oak Mountain and along the ridgeline is not currently in conservation but is described for conservation by the MSHCP. This is where some of the best Quino habitat is located. Almost annually we have Quino detections in this area from the Biological Monitoring Program biologists and from the Bureau of Land Management (BLM).

Core Area Definitions and Species Objectives: Adding the San Bernardino National Forest to our Core Areas designation should be considered for this species. Quino have been observed at two of our sites in this area, SBNF and Horse Creek, during several survey seasons.

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Appendix A. Butterfly and Moth Species, Listed by Family, Observed During the 2021 Quino Survey Effort

Swallowtails (Papilionidae)
- Western tiger swallowtail (*Papilio rutulus*)
- Pale swallowtail (*Papilio eurymedon*)
- Anise swallowtail (*Papilio zelicaon*)

Whites and Sulphurs (Pieridae)
- Checkered white (*Pontia protodice*)
- Becker's white (*Pontia beckerii*)
- Orange sulphur (*Colias eurytheme*)
- Desert orangetip (*Anthocharis cethura*)
- Sara orangetip (*Anthocharis sara*)
- California dogface (*Zerene eurydice*)
- Southern dogface (*Zerene cesonia*)
- Sleepy orange (*Abaeis nicippe)*

Coopers, Hairstreaks, & Blues (Lycaenidae)
- Great copper (*Lycaena xanthoides*)
- Gray hairstreak (*Strymon melinus*)
- California hairstreak (*Satyrium californica*)
- Mountain mahogany hairstreak (*Satyrium tetra*)
- Brown elfin (*Callophrys augustinus*)
- Perplexing hairstreak (*Callophrys perplexa*)
- Loki juniper hairstreak (*Callophrys gryneus loki*)
- Silvery blue (*Glaucopsyche lygdamus*)
- Marine blue (*Leptotes marina*)
- Acmon blue (*Plebejus acmon*)
- Lupine blue (*Plebejus lupinus*)
- Boisduval's blue (*Plebejus icarioides*)
- Echo azure (*Calastrina echo*)
- Ceraunus blue (*Hemiargus ceraunus*)
- Bernardino blue (*Euphilotes bernardino bernardino*)
- Western tailed-blue (*Cupido amyntula*)
- Western pygmy-blue (*Brephidium exile*)

Metalmarks (Riodinidae)
- Behr's metalmark (*Apodemia virgulti*)

Brushfoots (Nymphalidae)
- Gabb's checkerspot (*Chlosyne gabbii*)
- Quino checkerspot (*Euphrydryas editha quino*)
- Chalcedon checkerspot (*Euphydryas chalcedona chalcedona*)
- American lady (*Vanessa virginiensis*)
- Red admiral (*Vanessa atalanta*)
- Mourning cloak (*Nymphalis antiopa*)
- California sister (*Adelpha bredowii*)
- Coronis fritillary (*Speyeria coronis*)
- Mylitta crescent (*Phyciodes mylitta*)

Skippers (Hesperiidae)
- Funereal duskywing (*Erynnis funeralis*)
- Propertius duskywing (*Erynnis propertius*)
- Pacuvius duskywing (*Erynnis pacuvius*)
- Northern white skipper (*Heliopetes ericetorum)*
- Juba skipper (*Hesperia juba*)
- Fiery skipper (*Hylephila phyleus*)

Night Moths (Noctuidae)
- Unidentified night moths

Geometer Moths (Geometridae)
- Unidentified geometer moth

Sphynx Moths (Sphingidae)
- Unidentified Sphynx moth

Appendix B. Core Area and Satellite Occurrence Complex Detections and Average Precipitation (Inches) from 2008-2021

Core Area	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Lk Mathews/Estelle/ Harford Springs	0†	0	0	0	0	--	--	--	--	--	--	--	0	--
Warm Springs Creek	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Johnson Ranch/Lake Skinner	1	1	1	1	1	1	0	1	1	1	1	1	1	1
Oak Mountain	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Wilson Valley	1	0	1	1	0	1	0	0	0	0	0	0	0	0
Sage	1	0	1	0	0	1	0	1	0	1	1	0	0	0
Silverado/Tule Peak	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Satellite Occurrence Complex (Non-Core Area)														
SBNF	1	0	1	1	1	1	0	1	1	1	0	1	1	0
Cactus Valley	0	0	1	0	0	0	--	--	--	0	1	0	0	0
Anza Valley	--	--	--	--	--	0	--	--	--	--	--	--	--	--
Aguanga	--	--	--	--	--	--	--	--	--	--	--	--	0	0
Estimated annual precipitation of southwestern Riverside County (NOAA, 2021)	17.5”	15”	20"	30”	15”	12.5”	12.5”	15”	16.25”	30”	8.7”	25”	15.0”	12.5”

†no detections = 0, detections = 1, no surveys = --